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Heart Rate Variability (HRV) Analysis A Methodology for Organizational Neuroscience — Source link

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Abstract

Recently, the application of neuroscience methods and findings to the study of organizational phenomena has gained significant interest and converged in the emerging field of organizational neuroscience. Yet, this body of research has principally focused on the brain, often overlooking fuller analysis of the activities of the human nervous system and associated methods available to assess them. In this paper, we aim to narrow this gap by reviewing heart rate variability (HRV) analysis, which is that set of methods assessing beat-to-beat changes in the heart rhythm over time, used to draw inference on the outflow of the autonomic nervous system (ANS). In addition to anatomo-physiological and detailed methodological considerations, we discuss related theoretical, ethical, and practical implications. Overall, we argue that this methodology offers the opportunity not only to inform on a wealth of constructs relevant for management inquiries, but also to advance the organizational neuroscience research agenda and its ecological validity.

Keywords: affect and cognition; autonomic nervous system (ANS); ecological validity; heart rate variability (HRV); neuroscience methods; neurofeedback; organizational neuroscience.

Heart Rate Variability (HRV) Analysis: A Methodology for Organizational Neuroscience

In recent years, organizational and management research has looked with increasing attention at the use of neuroscience in its domains, with a growing number of contributions converging in the emerging field of organizational neuroscience (ON; Becker, Cropanzano, & Sanfey, 2011; Senior, Lee, & Butler, 2011)[1]. Notwithstanding this interest, both the theoretical works and the few empirical studies seen thus far in ON have predominantly focused on the brain and on brain-imaging techniques, such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) (e.g., Bagozzi et al., 2013; Waldman, Balthazard, & Peterson, 2011). Conversely, there has been an overall lack of research exploring insights derived from analysis of the fuller activities of the human nervous system and embracing the wide-ranging methodological toolkit that modern neuroscience can insert into organizational scholarship.

Notably, measurements associated with the activity of the autonomic nervous system (ANS)[2], like cardiovascular measures, electrodermal activity (EDA or galvanic skin response), and blood pressure variation, have been largely disregarded in ON thus far. This knowledge shortage is highly surprising because these are known outcomes of the nervous system's activities and/or responses—thus "neuroscience measures" in their own right—and certainly relevant for quantitative observations of psychological and social phenomena pertaining to organizational studies. For one, cognate disciplines like social psychology and social neuroscience (e.g., Cacioppo, 1994; Cacioppo & Sandman, 1978; Cacioppo, Tassinary, & Berntson, 2000; Cameron, 2002) have already established the value of examining ANS responses to indicate variations in several constructs, ranging from emotions (e.g., Quintana, Guastella, Outhred, Hickie, & Kemp, 2012) to social interactions (e.g., Blascovich, Mendes, Hunter, Lickel, & Kowai-Bell, 2001). Indeed, the ANS responses measured can represent antecedents of conscious awareness (Bechara, Damasio, Tranel, & Damasio, 1997), map differences amongst emotions (Levenson, 1992), and also inform on complex cognitive processes, such as reasoning about social dilemmas (Grossmann, Sahdra, & Ciarrochi, 2016), which may be difficult to fully appreciate by means of more conventional research methods.

Moreover, and this is of particular interest for the managerial literature, the associated ANS methods allow for a constructive address of some conceptual and practical challenges currently

limiting the advancement of ON. Speaking generally, they require simpler analyses than those employed in brain-imaging methods (e.g., fMRI, EEG); involve relatively inexpensive and readily accessible devices; and supply real-time variables of dynamic systems, also offering support to ecologically valid research.

In this paper, we systematically introduce readers to one of these ANS methods, with the aim both to enrich the current methodological apparatus of organizational scholars, as well as to contribute to the extant conversation in ON. We specifically focus on heart rate variability (HRV) analysis—here defined as the assessment of the neurophysiological phenomenon of variation in the time interval between consecutive heartbeats, used to draw inference on the outflow of the ANS complex dynamics. We purposely concentrate on HRV analysis because, differently from akin ANS methods, this methodology has lacked exposure in the managerial and ON literature thus far (cf. Becker & Menges, 2013; Massaro, 2016). Furthermore, in the past few years, this approach has seen impressive methodological advances (Kuusela, 2012). Yet, the often-imprecise use of its metrics (as reported in Pagani, Lucini, & Porta, 2012) has also highlighted lack of coherent foundation for this method to fulfil its potential, thereby calling for an updated review.

This work unfolds its contribution as follows. We begin by outlining the necessary anatomo-physiological features of the ANS and its associations with the control of the cardiovascular system. We then offer a thorough methodological appraisal on HRV analysis, integrating this with an explanation of related practicalities, developments, limitations, and ethical considerations. We then turn our discussion to introduce some illustrative cases in which HRV analysis can be applied to advance management theory and practice. Finally, we argue that this methodology not only is well-suited to complement more traditional organizational research techniques, but also holds the potential to rapidly become an effective, shared, and relatively easy-to-use tool to extend the current ON agenda.

Throughout this paper, we have sought to use jargon-free language. However, because the key aims of this work are to familiarize readers with the features of rigorous HRV analysis and enable them with appropriate terminology, at times we have necessarily used a more technical lexicon. We recognize that this may appear as a bold attempt not yet frequently used in organizational

neuroscience. However, we believe that promoting accurate thinking and writing can help clear up some of the current murkiness and skepticism toward neuroscience and its methods amongst organizational scholars. For this reason, we have developed our work by both thoroughly describing the scientific terms used and including supporting material.

Organizational Neuroscience and the ANS: An Overview

From a layperson's perspective, neuroscience is often equated with the science of the brain. While the brain is undeniably the most attention-grabbing and least understood organ of our body, this equivalence entails a simplification often naïvely taken up by management researchers. Indeed, as Ward, Volk, and Becker (2015) have recently pointed out, despite several perspectives having appeared in ON thus far, they all have as a common element the "*brain*-level of analysis" (p. 19; italics added). This convergence is not surprising since the great majority of current ON research has either centered on discussions about the cerebrum and its regions and components (e.g., Becker et al., 2011) or empirically employed brain-imaging tools (e.g., Bagozzi et al., 2013; Balthazard, Waldman, Thatcher, & Hannah, 2012).

Yet, a more complete appraisal of neuroscience suggests that neuroscience is "the scientific study of the nervous systems and their role in behavior" (Society for Neuroscience, 1969). The human nervous system is the system that coordinates voluntary and involuntary actions and transmits signals between different parts of our body, and of which the brain is just one fundamental element. Therefore, if we seek to leverage neuroscience to enrich knowledge of organizational phenomena, we may as well actively include experimental investigations, theoretical accounts, and methodology that look beyond the brain.

Particularly in recent years, there has been growing momentum in autonomic neuroscience, a disciplinary field focusing on the involuntary part of the nervous system, the ANS (for a primer on the ANS, see e.g., Robertson, Biaggioni, Burnstock, & Low, 2012), with HRV emerging as one of its key multidisciplinary components (Kuusela, 2012). This body of work has not only provided important information on several behaviors and socio-psychological constructs but, as we shall see, also implicitly increased opportunities to further organizational research. Thus, we believe that the

inclusion of research based on measurements of ANS activities could (and should) become an integral part of the organizational neuroscience program.

In order to provide a fertile background for reviewing HRV analysis and exploring related research opportunities, and given the complexity of the human nervous system, we now consider the main characteristics of the ANS and of its association to cardiac activity.

Anatomo-physiological Foundations of HRV Analysis

The human nervous system is composed of the central nervous system (CNS)—the brain and the spinal cord—and the peripheral nervous system (PNS), which connects the CNS to other parts of the body. Of particular interest for our purpose is an anatomical division of the PNS, the autonomic nervous system, which innervates internal organs and whose activity is independent from our voluntary control (for a review on the ANS, see Gabella, 2012). Functionally, the ANS involves both peripheral and central elements: Ganglia (i.e., groups of nerve cell bodies) and nerves spread through the body, while several centers and nuclei (i.e., large aggregates of neurons) are located in the CNS (for a review on the central autonomic nervous system, see Saper, 2002). The central component is distributed throughout the neuraxis (i.e., the axis of the CNS) and has a primary role in instant control of visceral function, internal regulation, and adaptation to external challenges. The peripheral component consists of nerves that develop from the brainstem (i.e., the posterior part of the brain that connects with the spinal cord) and the spinal cord to reach the autonomic ganglia, and from there, other nerves connect with the peripheral tissues, including the cardiac muscle.

Speaking generally, the ANS helps regulate several bodily functions, such as cardiac activity, respiration, vasomotor action, and reflex actions like coughing and sneezing, among others. These phenomena are influenced by two complementary activities of the ANS (Table 1): sympathetic and parasympathetic[3].

---{Insert Table 1 About Here}---

Sympathetic activity is primarily connected to the preparation of the body for response to action in demanding and/or worrying situations, commonly known as the “fight or flight” response. On the other hand, parasympathetic activity functions under more restful situations and counteracts the effects of sympathetic activity to reinstate and keep the body in a balanced state (i.e., *homeostasis*,

from the Greek: *homeo*, similar; and *stasis*, steady). Parasympathetic activity is usually identified as the "rest and digest" or "feed and breed" response. We believe it is important to emphasize that the ANS is always working, and under normal situations, it maintains a dynamic and complex state of equilibrium between these two activities.

Notably, while our heart is an organ that can operate and respond independently of neural control systems thanks to its pacemaker tissues (Franchini & Cowley, 2004), its activities are strongly influenced by these ANS functions. Indeed, the heart is innervated by both sympathetic and parasympathetic nerves, as well as by an intrinsic complex system of nerves (Armour, Murphy, Yuan, MacDonald, & Hopkins, 1997). Altogether, this autonomic activation influences the heart rate, conduction, and hemodynamic, as well as cellular and molecular properties of individual cells (Shen & Zipes, 2014). Speaking generally, parasympathetic stimulation, mainly through the action of the vagus nerve, slows heartbeat variation. Conversely, heartbeat variation increases in response to the sympathetic modulation, determining chaotic fluctuations in recordable signals (Lombardi, 2000). This modulation occurs because the ANS innervates the cardiac pacemaker tissues (i.e., sino-atrial and atrio-ventricular nodes of the heart) responsible for initiating and spreading electrical signals during each heart cycle, making them subject to the paired and opposed ANS influences just described[4].

The heart of a normal healthy individual is constantly subject to these activities and maintains a natural status of balance (often referred to as sympathovagal balance; see e.g., Lombardi, Malliani, Pagani, & Cerutti, 1996). Importantly, these features also reflect a person's ability to react, for instance, to external threats and/or internal emotional changes and to restore homeostasis once the eliciting situation is gone.

Therefore, we can readily recognize that the ability to measure variations in several heart activities, including rhythm and rate, can offer explanatory "proxies" to appreciate the "upstream" activity of the nervous system as well as people's psychological states and behavioral responses. This information in turn can offer important insights for organizational research. For one, work stress strongly influences the overall heart activity (for a meta-analysis and a discussion on the direction of the effect in several HRV features, see Castaldo et al., 2015). Similarly, heart rate is positively

correlated with demanding cognitive and semantic tasks (e.g., Carroll, Turner, & Hellawell, 1986; Mulder, 1992).

Yet, to make the most of this physiological information, it is essential to better understand what the heart rate actually represents and how we can accurately measure and analyze its variations.

Measuring Heartbeats and Heart Rate

If asked how to assess our heartbeat, we may intuitively think about checking our pulse or using an electrocardiographic device. Indeed, an electrocardiogram (ECG or EKG) is a representation of the electrical activity of the heart, projected on the skin surface, along standard references (Kligfield et al., 2007). A normal ECG waveform (i.e., the ECG graph), as shown in Figure 1, is comprised of several complexes, known as P, Q, R, S, and T waves[5]. The deflections (i.e., the distances between reference points on those complexes) and complexes have been widely standardized and studied (Kligfield et al., 2007; Wilson, Macleod, Barker, & Johnston, 1934). The complexes denote various stages of the heart cycle. The main peak of the waveform (i.e., the QRS complex) represents the depolarization of ventricles, which also corresponds to ventricular contraction, while T represents repolarization and relaxation (for an introduction on the heart's cycle, see Rushmer & Blackmon, 1970).

---{Insert Figure 1 About Here}---

ECG data can be acquired from several configurations. In conventional ECG setups, electrodes are placed on a subject's wrists and/or ankles (for a review on standard ECG configurations, see Davis, 1992); recent portable ECG devices also allow torso placement. The latter is generally preferable in organizational research because it allows research subjects to move more freely in their environment (see also the section of this paper on "Practicalities and Novelties").

The acquisition of ECG data is the first passage needed to determine several measures of heart activity used to infer information on the outflow of the ANS (Jessup et al., 2009; Task Force, 1996). One of these time-based measures is the heartbeat period, the period between two consecutive heartbeats, also called inter-beat interval or RR, which is the time the heart needs to complete one cycle and is fundamental to compute HRV (Figure 1). RR (ms) can be expressed as the time difference between consecutive R peaks, as follows:

$$RR_n = t_n - t_{(n-1)} \qquad \text{(Eq. 1)}$$

The graph representing beat-to-beat RR intervals is called the RR tachogram. The mean value of RR intervals is called *AVNN* (ms) and is computed as:

$$AVNN = \frac{1}{N}\sum_{j=1}^{N} RR_j \qquad \text{(Eq. 2)}$$

with RR_j denoting the value of the j'th RR interval and *N*, the total number of successive intervals. RR (ms) is also the inverse of the instantaneous heart rate (iHR):

$$RR_n = 1/iHR_n \qquad \text{(Eq. 3)}$$

iHR is based on a single inter-beat interval and thus can vary between successive intervals.

The count of RR intervals in one minute (i.e., 60 s or 60,000 ms) gives the mean heart rate (hereafter, HR). HR is what we commonly refer to as our "heartbeat" in our everyday language and is usually expressed in beats per minute (bpm). An adult's normal resting HR ranges from 60 to 100 bpm. Speaking generally, a lower HR at rest implies more efficient heart function and better cardiovascular fitness. For example, a professional athlete might have a normal resting HR close to 40 bpm (Aubert, Seps, & Beckers, 2003).

Basic HR measures have already been used effectively in social and behavioral sciences (Katkin, 1985; Schandry & Bestler, 1995). For instance, attempts to evaluate self-perception have focused on the cardiovascular system because our "heartbeats" (i.e., HR) are discrete physiological events measurable with little effort (e.g., Katkin, Blascovich, & Goldband, 1981). Heartbeat self-detection[6] is a widely employed research strategy (e.g., Brener, Liu, & Ring, 1993; Katkin, 1985; Schandry, 1981), and over the years it has been used to investigate important constructs, such as social phobia (Antony et al., 1995). Likewise, psycho-physiological theories of emotions have historically suggested that self-perception of visceral activity is a crucial component of our emotional experience (Larsen, Berntson, Poehlmann, Ito, & Cacioppo, 2008; James, 1884). Heartbeat detection has offered support to examine, for instance, the relationship between self-report of emotional experience and individual differences in self-perception (e.g., Wiens, Mezzacappa, & Katkin, 2000), which is another main feature of the organizational life (e.g., Yammarino & Atwater, 1993).

Lately, neuroscience research has investigated further the relationship between cardiac activity and self-awareness (Garfinkel et al., 2014; Park, Correia, Ducorps, & Tallon-Baudry, 2014; Salomon et al., 2016), which is a fundamental aspect of managerial cognition (Church, 1997) and leadership (Van Velsor, Taylor, & Leslie, 1993), among others. There are mounting indications that interoceptive signals, which carry knowledge about the internal state of our body (see e.g., Tsakiris, Tajadura-Jimenez, & Costantini, 2011), influence self-awareness. For instance, Salomon et al. (2016) showed that cardiovascular signals regulate awareness of visual stimuli. By using fMRI, they explained that visual elicitation occurring at a subject's own cardiac frequency requires longer to access cognizance. Fascinatingly, they found that the insula responds to this phenomenon even when the stimuli are made invisible to the subjects, suggesting the existence of a neuro-perceptual suppression mechanism regulating human awareness. Along these lines, Park et al. (2014) showed that detection of faint visual stimuli is associated with the amplitude of the heartbeat-evoked response. They contend that heartbeat signals, together with other visceral and proprioceptive information, may support a "neural subjective frame"—neural maps of the internal state of the body from which the first-person experience (i.e., one's overall sense of "I") is created (Park & Tallon-Baudry, 2014, p. 1).

Finally, simplified HR detection procedures have also found growing space in practice, such as in the development of coaching and leadership programs centered on basic forms of biofeedback or neurofeedback—conditioning protocols that seek to train people to change their behavior by monitoring target physiological processes (Lehrer et al., 2003).

Despite the increasing potential for these avenues, scholars have also called for the use of more refined analytical methods, based on complex computation of ECG signals, both in research and in practical applications (Pagani et al., 2012). This also allows for a more accurate understanding of the outflow of the ANS, enabling a superior appreciation of the psychological, social, and behavioral constructs of interest. To this end, we now describe one of the most promising and reliable methodologies based on the assessment of ECG data: heart rate variability analysis.

Heart Rate Variability Analysis

Recently, several techniques grounded on the processing of ECG data have been used as indexes of ANS modulation on the heart. Among them, the analysis of heart rate variability has

emerged as one of the most rapid and non-invasive methods used to obtain reliable and reproducible information on the autonomic modulation of heart rate (Sztajzel, 2004; Parati, Mancia, Di Rienzo, & Castiglioni, 2006).

Overall, HRV represents the fluctuation between intervals of consecutive heartbeats resulting from the non-stationary autonomic influence[7] (Montano et al., 1994; Task Force, 1996). Moreover, HRV has a complex chaotic structure involving components non-linearly related to each other (Lombardi, 2000). By describing how HRV analysis can be performed, we seek to detangle such complexity.

A Historical Rationale

In 1934, Rosenblueth and Simeone (1934) had already demonstrated that sympathetic and parasympathetic influences on heart automaticity can be expressed by the product of their separate effects. Heart rate (HR) can indeed be expressed as:

$$HR = m(n)HR_0 \qquad \text{(Eq. 4)}$$

in which HR_0 is the intrinsic heart rate (i.e., HR under complete pharmacological blockade); m is a factor representing sympathetic acceleration ($m \geq 1$); and n, a factor representing vagal deceleration ($n \leq 1$). The product mn (that is, HR/HR_0) can also be regarded as the sympathovagal balance (see, Bootsma et al., 1994; Montano et al, 1994). This balance is either ≤ 1 or ≥ 1, respectively, under conditions of parasympathetic (i.e., vagal) or sympathetic predominance.

Yet, pharmacological blocking of the nervous system influences was needed to experimentally determine HR_0. It was thus clear that a non-invasive procedure to determine HR was preferable. As a consequence, HRV analysis was postulated to offer a non-invasive option to study cardiac autonomic activity. This approach largely leverages differences in the latency of the sympathetic and parasympathetic actions, which results in different "speeds" (i.e., frequencies) in which the oscillations in the heart rate are produced (Appelhans & Luecken, 2006; Task Force, 1996) (see also the section of this paper on "Inferring Information from HRV Analysis").

From these earlier insights, research on HRV rapidly took off in the 1970s (e.g., Lacey & Lacey, 1970; Lacey & Lacey, 1978), suggesting that changes in cardiovascular functions also facilitate or inhibit cortical processing. For one, the classic work by Lacey & Lacey (1978, p. 99)

suggests a "two-way communication between the heart and the brain." These scholars showed that the greater the cardiac deceleration, the faster the individual's reaction time; cardiac deceleration would then match attention and preparation for action.

Successively, Akselrod et al. (1981) introduced a refined method of investigating heart rate fluctuations to quantitatively evaluate inter-beat cardiovascular control: the spectrum analysis. Since then, the use of HRV analysis as a neuroscience method has blossomed and further benefited from refined computational approaches to isolate changes in heart rate due to parasympathetic, sympathetic, or a combination of both activities. This knowledge has in turn begun to inform an increasing number of social, psychological, and behavioral topics, many of which, as we shall see, are relevant for organizational research.

Computing HRV: An Outline

Computing HRV essentially means computing a time series that describes the temporal variation in consecutive heartbeat intervals.

---{Insert Figure 2 About Here}---

As summarized in Figure 2, the overall methodological process entails several steps, including: acquisition of ECG signal (i.e., a continuous measure holding information on HR) with a high-resolution frequency, usually of 500-1000 Hz (e.g., Berntson & Stowell, 1998); correction of abnormal beats and artifacts; computation of inter-beat intervals (i.e., RR and NN series); and computation or extraction of HRV features, the actual HRV analysis. Figure 3 presents an illustrative graphical breakdown of HRV extraction from a digitized ECG signal based on real data.

---{Insert Figure 3 About Here}---

Stable detector algorithms (e.g., Pan & Tompkins, 1985) are deployed to perform these computational steps and obtain graphs like those shown in Figure 3. While the study of detection algorithms represents an exciting and flourishing area of research, this coverage falls beyond the scope of this work. Yet, speaking generally, these algorithms include two key components: extraction of the characteristics of the ECG signal, and waveform classification and recognition. Fortunately for novice researchers, there are several software packages available to assist with these tasks (see also the section "Software" of this paper).

R Peaks Detection and Annotation

To extract useful information about heartbeat changes from an ECG, it is necessary to undertake some pre-processing steps. In other words, once digitized, an ECG signal—which is a non-stationary wave (i.e., its frequency content and period change over time)—is processed so that a series of inter-beat intervals can be extracted and eventually corrected for abnormal beats and other artifacts (Kamath & Fallen, 1995).

To this end, it is essential to observe and correctly annotate all the R peaks (or R spikes) on the length (for an explanation on length, see the section "Computing HRV Analysis" of this work) of the ECG signal under investigation (Task Force, 1996). R is the most outstanding characteristic waveform in the ECG. It is closely related to the discrimination of a normal ECG cycle and usually has the highest or lowest value in the QRS complex. Thus, the R annotation is a valid means to extrapolate heartbeat instances from an ECG wave and, in turn, compute the inter-beat period (for a graphical illustration, see Figure 3 [b] and [c]).

Following R peaks detection, a morphological analysis of the QRS complex is performed to understand whether the detected peaks are normal (Acharya et al., 2004). Figure 4 simplifies apparent differences between normal and abnormal peaks.

---{Insert Figure 4 About Here}---

If the peaks are abnormal, it is necessary to understand whether this is due to physio-pathological causes (e.g., arrhythmia or ectopic beats, a condition in which the beats arise from fibers outside the SN node); errors in the detection algorithms; or confounding factors and experimental artifacts (e.g., a subject's abrupt movement during the ECG registration). While this computational procedure is embedded in several software packages, it is also good practice to perform a "manual" quality control on random segments of the ECG wave to ensure the reliability of the algorithm used.

Once confirmed that there are no clinical conditions affecting the subjects, in the presence of abnormal R peaks, researchers can either repeat the R peak detection process, eventually using a different algorithm (e.g., one more resistant to artifacts), disregard the ECG segments containing abnormal R peaks (hence, the importance to record signals beyond the chosen length of analysis; see also the section "Computing HRV Analysis"), or decide to repeat the entire ECG acquisition.

After the abnormal peaks have been excluded, it is then possible to compute the inter-time between consecutive heartbeats: the RR series. Note that despite the extraction of HRV features being applied to a corrected series—rather than to factual normal-to-normal heartbeat intervals—this resulting series is usually called the NN series or Normal-to-Normal series.

Validation: Errors and Quality of the Signal

Biological and physiological signal analyses are often subject to several sources of uncertainty throughout the process. Notably, the ECG device used can affect the quality of the time series recorded (for recommended features of ECG devices, see the section "Tools, Costs, and Maintenance"). Likewise, multiple physiological parameters (e.g., blood pressure, pulse rate, and thoracic sounds) can introduce uncertainty in the R peak detection (Acharya, Joseph, Kannathal, Lim, & Suri, 2006). For instance, mechanical properties (i.e., elasticity or stiffness) of the arterial vessels can influence the estimation of the QRS complex position in time. If not addressed, this uncertainty may propagate to the whole HRV analysis and mask the real effect of the experiment under investigation, reducing the significance of the study. To tackle these concerns, we recommend the use of stable detection algorithms, appropriate sample sizes, and quality checks on R peaks annotation.

In particular, quality assurance on the acquired signal is fundamental to extract meaningful HRV features. While there are several approaches available, the most widely used consists of calculating the percentage of NN intervals over the total number of RR initially detected, as indicated in Eq. 5.

$$X = (NN/RR)100 \qquad \text{(Eq. 5)}$$

If this value falls below 90%, it is good practice to discard the entire signal. Checking this ratio is paramount to ensure analytical rigor because a low quality signal may produce poor, if not erroneous, findings. Other routine controls involve assessing the slower periodic phenomena under observation and the minimum time distance between two consecutive significant periodic phenomena. Note that performing these controls requires advanced knowledge of signal processing (for a discussion, see e.g., Akay, 2012).

Finally, once the NN series has been thus qualified, it is possible to calculate the HRV series to be analyzed by subtracting from the NN series its mean. The resulting set of inter-beat intervals is then used to compute two possible classes of HRV analysis, as described below.

Computing HRV Analysis

A key preliminary step in computing HRV analysis involves the choice of the time length of the signal to analyze (i.e., the choice of the excerpt or segment). The length of the excerpts is chosen according to the phenomenon under observation, the experimental conditions, the research question of the study, as well as the research subjects' personal circumstances and physiological cycles (e.g., circadian patterns and menstrual cycles; see e.g., Lombardi, 2002). The literature considers three main standardized lengths (Task Force, 1996): (a) long-term, which refers to nominal 24-hour HRV excerpts; (b) short-term, which refers to five-minute excerpts; and (c) ultra-short-term, which refers to excerpts under five minutes.

Note that an excerpt does not necessarily correspond to the full duration of the signals recorded in an experiment. For instance, researchers may acquire several hours of data; yet, they can also perform a complete HRV analysis on five-minute excerpts only. A general indication for ON investigations is to conduct analyses of at least 250 seconds (i.e., nominally five minutes), preceded by another five-minute stabilization period (i.e., the period "at rest"). Moreover, a general rule of thumb is that data obtained from short-term segments are best processed with frequency domain methods; time domain analyses are better suited to analyze long-term recordings.

Indeed, there are several available approaches to compute HRV analysis (Acharya et al., 2006; Task Force, 1996). These are usually categorized into two broad groups (Table 2): (a) linear measures, which can be analyzed either in the time domain or in the frequency domain; and (b) non-linear measures. We introduce their overall characteristics below. We also present a formal mathematical approach in the "Supplementary Material" section of this paper.

---{Insert Table 2 About Here}---

HRV time domain estimates. The simplest HRV analyses are the time domain estimates. These are descriptors of the NN time series and can be divided into statistical and geometrical methods.

Statistical measures are standard deviations of the RR intervals, which reflect the overall variation within the RR interval series (Copie et al., 1996; Stein, Bosner, Kleiger, & Conger, 1994; Ziegler, Piolot, Strassburger, Lambeck, & Dannehl, 1999). The most popular approaches are summarized in Table 2.

The geometrical measures are relatively insensitive to the analytical quality of the RR series and are often unfit to assess short-term data. The most common are the HRV triangular index—the integral of the density distribution (computed as the total number of NN intervals) divided by the maximum of the density distribution of NN intervals; and the baseline width of the distribution, measured as the base of a triangle approximating the NN interval distribution (for discussion, see Copie et al., 1996).

HRV frequency domain analyses. Frequency domain analyses require a more sophisticated knowledge of the HRV signal because they rely on the estimation of power spectral density (PSD)—the description of how the power of the signal is distributed over frequency (Orini, Bailón, Laguna, & Mainardi, 2007).

These measures can be computed using: (a) parametric methods, which can be deployed when the power of the HRV in time is regularly distributed (i.e., HRV is sufficiently stationary); and, (b) non-parametric methods, which perform better when the signal changes significantly during the observation (i.e., non-stationary HRV).

The non-parametric methods are generally based on Fast Fourier Transformation (FFT), which offers simplicity in computation and high processing speed, despite suffering from spectral leakage (i.e., the situation in which a signal that has one or two main frequency components shows more components than expected even if there is no noise in the signal). Contrarily, the parametric methods—the most widely used being the autoregressive model (AR)—do not have issues of spectral leakage and offer easier post-processing of the spectrum (Mendez et al., 2007).

HRV non-linear measures and rhythm pattern analyses. A separate category of HRV analysis involves non-linear approaches. Simply put, these involve the quantification of the "chaos" in the heart rhythm or the quantification of the behavior of HRV patterns over different time scales (i.e., a few minutes vs. 24 hours). The most common non-linear methods applied to HRV analysis are:

Poincaré plot (Melillo, Fusco, Sansone, Bracale, & Pecchia, 2014), approximate entropy (Pincus, 1991), sample entropy (Richman & Moorman, 2000), correlation dimension (Carvajal, Wessel, Vallverdú, Caminal, & Voss, 2005), detrended fluctuation analysis (Penzel, Kantelhardt, Grote, Peter, & Bunde, 2003), and recurrence plot (Trulla, Giuliani, Zbilut, & Webber, 1996).

Despite the higher computational complexity required, these approaches have recently proven superior in quantifying and mapping non-linear and chaotic ANS activities, as well as in correlating HRV signals to precise psychological states of research subjects (Melillo, Bracale, & Pecchia, 2011). Importantly, when applying non-linear methods, researchers must ensure low signal-to-noise ratio, accurate estimation of high-frequency spectrum in short-time recordings, and low variability of signals (Pecchia, Melillo, Sansone, & Bracale, 2011).

Inferring Information from HRV Analysis

Research has shown that both time domain and frequency domain HRV indexes can provide useful information on the ANS modulation. For instance, time domain measures of standard deviation, coefficient of variance, and mean successive difference positively correlate with vagal tone at rest (Hayano et al., 1991). Likewise, bands representing main oscillatory components of the HRV power spectrum (i.e., the distribution of frequency components of the signal) yield explanatory insights on the ANS outflow. The most used components are: very low frequency (VLF: ≤ 0.04 Hz); low frequency (LF: $0.04 - 0.15$Hz); and high frequency (HF: $0.15 - 0.4$Hz). The power of high-frequency bands primarily reflects efferent vagal, thus parasympathetic, influence; while the power of LF bands is associated with vagal, sympathetic, and baroreflex mechanisms, and is largely dependent on the context (Task Force, 1996).

Notwithstanding ongoing debates (Billman, 2013; Malliani, Pagani, Lombardi, & Cerutti, 1991; Parati et al., 2006; Task Force, 1996), researchers have argued that the ratio of LF to HF power (LF/HF) can possibly represent an informative index of the sympathovagal balance. This value would characterize relative shifts toward either parasympathetic or sympathetic dominance on cardiac function, offering a simple means to extract information on ANS activity from HRV (Malliani, Lombardi, & Pagani, 1994). Generally speaking, a low LF/HF ratio is believed to reflect greater parasympathetic activity than sympathetic; however, this value is often altered due to a greater

depression of LF power than of HF power (Shaffer, McCraty, & Zerr, 2014). Indeed, the relationship between sympathetic and parasympathetic modulations in generating LF bands is non-linear and contingent on experimental conditions (Billman, 2013). Therefore, inferences on ANS outflow derived from frequency domain outputs, and LF/HF values in particular, should be always interpreted with caution, especially in the presence of short-term excerpts.

Because of these reasons and the fact that both time and frequency domain methods have often led to converging results, we recommend ON researchers to always perform and evaluate their analyses with multiple HRV indexes. For one, as we shall now see, the use of non-linear methods has increasingly delivered more nuanced and robust information on the activity of ANS (Brennan, Palaniswami, & Kamen, 2001; Kamen, Krum, & Tonkin, 1996; Melillo et al., 2011).

In the following paragraphs we describe an illustration of an HRV analysis particularly suited to management research. Specifically, we look at the variation of several HRV indexes in relation to a healthy, normal subject performing a low vs. high mental load task (i.e., a low vs. high mental effort condition). Indeed, the organizational literature has recently shown an increasing interest in better understanding the role and mechanisms of mental effort in several domains, spanning from business ethics decision-making (Street, Douglas, Geiger, & Martinko, 2001) to entrepreneurs' mental models (Nambisan & Baron, 2013), as well as in practical managerial problems such as in air-control tasks (Yeo & Neal, 2008) and biofeedback on cognitive performance (Prinsloo et al., 2010).

We used a modified version of the Stroop Color-Word Interference Test (Stroop, 1935), which is highly sensitive in detecting low vs. high mental effort[8]. The data for our ad-hoc computation were collected with a BioPatch™ M3 device (Zephyr, Annapolis, USA), which is a wireless torso-placed bio-patch able to record ECG signals (see "Practicalities and Novelties" section). The recording was performed under standard conditions: in a quiet room at our institution, at a comfortable temperature, in the morning, while the subject was speaking, and minimizing other stimuli possibly affecting HRV.

In the following Figures we show in (a) information related to the baseline condition (i.e., the subject not performing any task); in (b) analyses associated with the low mental effort condition; and in (c) those linked to the more demanding mental effort situation. The analyses were performed using

Kubios© (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2009) and Matlab© and we controlled for potential artifacts and errors. We also refer readers to the "Supplementary Material" section of this paper for further details on the HRV indexes presented in the Figures.

---{Insert Figure 5 About Here}---

In Figure 5 we show the computed short-time RR series (5 minutes) of the subject performing the tasks. For the readers' benefit, in Figure 5 (a) we show the RR tachogram of the baseline, which is the RR series when the subject is "at rest." This is an important passage in every HRV study because it allows a means to assess the degree of homogeneity between subjects. In Figure 5 (b) we show the RR series of the subject when undertaking the low mental load task, and in Figure 5 (c) under the more demanding condition. Visually, we can easily appreciate that in Figure 5 (b), the average amplitude of the RR series is lower than in Figure 5 (a). However, in Figure 5 (c) the RR plot drops considerably if compared to Figure 5 (b) and presents a very depressed pattern.

---{Insert Figure 6 About Here}---

Because of the high variability of the RR series, it is challenging to infer reliable information on ANS outflow from these tachograms. Yet, the use of HRV analysis in inferring neuroscience information from these signals becomes clearer in Figure 6. Here, we show the derived histograms of the mean of the heart rate (bpm) and related time domain measures for the same subject. At rest the subject presents an average HR of 74±7 bmp. In Figure 6 (b) the average HR remains within a normal range, yet slightly shifted toward higher values than in Figure 6 (a) at 81±8 bmp. Remarkably, during the high mental effort condition the histogram is evidently shifted toward higher values (121±5 bmp). Simply put, as we can intuitively expect, the subject's heart rate is faster while performing the more demanding task.

Importantly, time domain measures are strongly influenced by changes in both sympathetic and parasympathetic activity, making them non-specific measures of autonomic modulation. In our case, one possible approach to quantitatively appreciate such influence is to calculate the root mean square of the successive differences between adjacent RR intervals (RMSSD). Generally speaking, this measure provides a fairly sensitive time domain measurement of parasympathetic activity.

Indeed, we can appreciate that the RMSSD values decrease from from 36 ms in Figure 6 (b) to 19 ms in Figure 6 (c).

---{Insert Figure 7 About Here}---

Moving forward, in Figure 7 we display the HRV analysis computed in the frequency domain. With an increase in mental effort, a strong inverse relationship between mental effort and HRV power is expected (e.g., Mukherjee, Yadav, Yung, Zajdel, & Oken, 2011). In Figure 7 (a) it is possible to observe a regular power spectrum distribution; in Figure 7 (b) the power spectrum shows a shift toward VLF (in pink), and a decrease in both LF (in blue) and HF bands (in yellow). In the more demanding condition (Figure 7 [c]) the HRV spectrum is significantly depressed, with the total power (1533 ms^2) reduced by almost four times if compared to that related to the low mental effort condition (5650 ms^2). For one, readers shall note that, although partly incomplete, HF power is a satisfactory measure of vagal cardiac control (Parati et al., 2006); indeed, we can appreciate how the HF power decreases in presence of a more demanding mental task (286 ms^2 in Figure 7 [c]).

It is however worth keeping in mind that these HRV features do not follow a Gaussian distribution; therefore, the interpretation of this type of data requires caution. As explained, the features in the frequency domain should be better observed together (i.e., total and relative power) and not by focusing only on the LF/HF ratio (i.e., notice the considerable drop in the LF power from Figure 7 [b] to Figure 7 [c]). At the same time, as shown in Figure 7 (a) and (c), similar, yet misleading, LF/HF values can be calculated through several changes in the numerator, in the dominator, or in both. Note that this consideration applies to both inter-subject and inter-groups study designs.

---{Insert Figure 8 About Here}---

Because of the non-linear interactions between sympathetic and parasympathetic activity, non-linear indexes have been recently proposed to offer a more accurate interpretation of the ANS outflow, as we show in Figure 8. Here we present the Poincaré plot analysis, which is the most commonly used non-linear method to assess the dynamics of HRV, and can offer reliable appreciation of changes in the ANS modulation (Brennan et al., 2001; Kamen et al., 1996; Melillo et al., 2011). It is a diagram in which each RR interval is plotted as a function of the previous RR interval: the values

of each pair of consecutive intervals define a point in the plot. This output can be evaluated in a qualitative way, by looking at the visual pattern, and quantitatively by calculating specific indexes (for the mathematical details see the "Supporting Material" section of this work). Simply put, the center of the plot represents the average RR interval length. As shown in Figure 8, the quantitative analysis involves drawing a circular area centered on the center of the plot and comparing points from two lines traversing through the center, for which the standard deviation is computed (i.e., SD1 and SD2). Generally speaking, these values decrease after the sympathetic stimulation with a concomitant change of shape in the plot, as we can appreciate both visually and numerically in the low mental effort (Figure 8 [b]) vs. high mental effort condition (Figure 8 [c]) of our example. Moreover, as a rule of thumb, the points are more scattered when vagal activity offsets the sympathetic one. This is distinctly shown in Figure 9, in which we present the same Poincaré plots from Figure 8 plotted on the same scale to better appreciate their changes in shape and magnitude.

---{Insert Figure 9 About Here}---

Overall, this demonstration provides a general illustration of the potential of HRV analysis for ON. Indeed, we detected variation in a subject's HRV indexes while performing a low vs. high mental load task, in an ecologically valid environment, and by using a wearable device able to record physiological signals related to subjective feelings of mental effort in real-time. Moreover, we offer some descriptive and quantitative inference on the status of the subject's ANS during the tasks.

However, we also believe it is important to remark that the inferences described here do not hold predictive nor causal information per se. Indeed, as we shall see in the following section "Research Design and Subject Sampling," to do so we would have necessarily required a fully developed empirical study, with accurate power calculation. However, this is beyond our methodological remit here.

We also would like to highlight that HRV responses are largely individual and, as we shall see, this increases the complexity of the study design, suggesting that HRV analysis in ON is more reliable when inferences are made at the group level. Toward this end, we refer readers to the related empirical works by Brennan et al. (2001) and Melillo et al. (2011), to further appreciate the sensitivity of HRV indexes.

Despite still being at their infancy, we also believe it is useful to mention that recent research is pushing the boundaries of HRV analysis toward provision of "personalized" physiological indicators, thereby opening novel opportunities to infer information on the ANS activity also for ON research. For one, Valenza, Citi, Lanatá, Scilingo, & Barbieri (2014) characterized four emotional states based on the circumplex model of affect (Posner, Russell, & Peterson, 2005) through the analysis of heartbeat dynamics. By using a probabilistic approach, they were able to propose a framework proficient in detecting emotions every 10 seconds, with an overall recognition accuracy of over 79%. The long-term potential of this research is fascinating; organizational studies on emotions soon may benefit from an unambiguous neuroscience assessment to precisely and speedily map one individual's emotions in any real-life setting.

We will provide other examples of the use of this and other HRV analysis methods for organizational research in the discussion of this work. For now, let us complete our methodological account by considering further important aspects of HRV analysis.

Further Methodological Considerations and Limitations

Confounding Factors

In addition to possible sources of errors already reviewed, there are several other endogenous (i.e., pertaining to the physiology and nature of a subject) and exogenous (i.e., external to a research subject's physiology or nature) factors that can result in a confounding HRV analysis. Considering these factors is a critical avenue in conducting and planning every HRV study. For instance, the relationship between HRV and vagal modulation has large inter-individual variation (Hautala, Kiviniemi, & Tulppo, 2009). Similarly, the co-modulation of various respiratory and circulatory factors occurs over multiple time-scales. It is also worth mentioning that HR can represent confounding effects for other neuroscience methods, such as fMRI (Murphy, Birn, & Bandettini, 2013), and HRV analysis can offer a means to control for these effects.

Respiration and physiological loops. Respiration influences HR. During inspiration, the influence of the vagus nerve is reduced and HR accelerates; the opposite occurs during expiration. Nonetheless, some scholars suggest that respiration frequency can be excluded from estimates of HRV (for a discussion see Denver, Reed, & Porges, 2007; Grossman & Taylor, 2007). Readers should

note that parasympathetic withdrawal largely modulates the HRV in the range of the LF spectral component.

Another physiological loop is that linked to blood pressure. Blood pressure oscillates cyclically with a period of about 10 seconds. Those changes, known as Mayer waves, are due to oscillations in the baroreceptor and chemoreceptor reflex control systems (Julien, 2006). As a result, the ANS senses the lowering blood pressure and activates the sympathetic system, causing HRV oscillations in the LF spectral component. Likewise, homeostasis of body temperature is the result of "closed-loop" physiological control systems, which affect vasodilatation and vasoconstriction. These mechanisms influence HRV cycles in the range of both low frequencies and very low frequencies (Okamoto-Mizuno et al., 2008).

Sex, age, and ethnicity. HRV is significantly lower in healthy women compared to men, and this finding is likely due to lower sympathetic activity in women (Ramaekers, Ector, Aubert, Rubens, & Van de Werf, 1998). Notice that other cofounding factors, such as menstrual cycle or body mass index (BMI), can reveal gender differences in HRV (Vallejo, Márquez, Borja-Aburto, Cárdenas, & Hermosillo, 2005).

Zhang (2007) demonstrated that the overall autonomic activity assessed with HRV consistently decreases from the age groups 10+ to 80+ years. Moreover, LF and HF decline as age increases. These findings are consistent with those by Bonnemeir et al. (2003), which show that in healthy subjects HRV decreases with age and variation is higher in females than in males. Research has also found that lower HF, LF, and LF/HF are consistently associated with older age in Caucasian Americans, but not in African Americans, and that young African Americans have a lower parasympathetic activity than Caucasian Americans (Choi et al., 2006).

Pathological conditions. Several pathological conditions affecting either the nervous system (e.g., brain damage and degenerative diseases), the heart (e.g., infarct and arrhythmia), or other bodily systems (e.g., renal failure and diabetes) can confound HRV analysis (Acharya et al., 2006). While these situations are unlikely to occur in organizational research—which ideally focuses on normal healthy subjects—it is worth keeping in mind that vagally and sympathetically mediated fluctuations may be independently affected by some disorders. For one, all normal cyclic changes in HR are

reduced in cases of depression (Sayar, Güleç, Gökçe, & Ak, 2002), which might represent a subtle condition to assess in ON research. Note that in subjects at risk of cardiovascular events, a persistent sympathetic activation and a reduced vagal tone could determine a marked reduction in the dynamic complexity of heart rate fluctuations; that makes heart periods less adaptable and less able to cope with the requirements of a continuously changing environment (Bigger et al., 1996; Goldberger, 1996).

Medications, smoking, and coffee. HRV can also be significantly affected, directly or indirectly, by various groups of drugs; therefore, the use of medications in research subjects should be appropriately evaluated when interpreting HRV indexes (Acharya et al., 2006). Research has also shown that smokers have increased sympathetic and reduced vagal activity. Smokers have reduced HRV under effect of ANS control (e.g., Hayano, 1990). Note that HRV also reduces with the acute intake of alcohol, suggesting sympathetic activation and parasympathetic withdrawal (Malpas, Whiteside, & Maling, 1991). Finally, coffee intake influences parasympathetic activity assessed via HRV analysis in healthy subjects (Monda et al., 2009). For instance, Hibino, Moritani, Kawada & Fushiki (1997) showed, compared to controls, a significant increase of high-frequency power in the HRV spectrum after caffeine intake, suggesting a related increase in vagal autonomic activity.

Movement and physical activity. Tulen and Man't'veld (1998) found that HR and LF, show significant increases when individuals change from a supine to sitting to standing posture. This is an important consideration for organizational research (e.g., think about assessing employees sitting at their desks or while standing). Regular physical activity strongly influences HR and HRV responses (Aubert et al., 2003). Trained subjects usually present a lower HR, making huge variations in HRV possible; the opposite occurs in untrained subjects. Also, the velocity of HR variation is different in fit vs. unfit individuals. The former can easily increase their HR in response to external or internal stimuli and also recover faster when those stimuli are gone. Verlinde et al. (1991) have compared the HRV of aerobic athletes with a control group and showed that the former have an increased power in all frequency bands.

Research Design and Subject Sampling

Because several factors can influence HRV signals, organizational researchers should take these influences into consideration to plan accurate studies. Notably, inclusion and exclusion criteria are the characteristics that the prospective subjects of a study must have (or not) to be included (or excluded) in the research. These must be fully detailed in every ON empirical study to ensure both research transparency and reproducibility.

Conventional inclusion criteria are age, sex, and race; these are normally planned at the recruitment stages, before the experiment is conducted. On the other end, exclusion criteria can also be considered during the experiment's development and its analytical phases. For instance: subjects' individual conditions (e.g., use of medications, presence of diseases, and level of physical activity); denial of informed consent; instances in which the individuals do not adhere to research protocol or withdraw; problems in interpreting the effects of an intervention on the HRV features; and all those cases in which it is not possible to acquire reliable information from HRV signals (i.e., signal errors, lack of quality, or strong influence of confounding factors).

Likewise, close attention has to be placed on the research design used in a study (Charness, Gneezy, & Kuhn, 2012). Speaking generally, in a within-subject design, each participant is exposed to more than one condition (e.g., a task with two different parameter values or stimuli). If the exposures are independent, it is possible to gain causal estimations by assessing how HRV features (or other dependent variables) varied when the experimental conditions changed. Under a between-subject design, each subject is exposed to one condition, and pending group assignment randomization, it is possible to infer causality by comparing the HRV features between the control and the experimental group (i.e., the group receiving an intervention). In order to minimize the heterogeneity related to HRV analysis, a within-subject design is usually preferred. However, it is also important to point out that these two forms of research design can be combined (e.g., Charness, Gneezy, & Kuhn, 2012).

Researchers should also report whether possible confounding factors were homogeneously present in both populations (or conditions), and, whenever appropriate, compute a statistical power analysis (for one example, see Melillo et al., 2015). This analysis helps estimate the number of subjects to be enrolled in a study to detect the effect of a given size with a given degree of confidence (or, under sample size constraints, it computes the probability of detecting the effect of a given size

with a given level of confidence). While influenced by the experimental conditions, an indicative "figure" for within-subject studies investigating psychological constructs relevant for organizational research is to include at least 40 subjects (on statistical power analysis, see e.g., Fritz & MacKinnon 2007; for an example of power analysis in HRV, see Castaldo et al., 2015).

A further note of particular relevance for research in organizational settings is that short-term recordings may fail to detect very low-frequency oscillations, while data from long-term recordings are more prone to be influenced by external alternating environmental conditions (Bigger et al., 1996; Goldberger, 1996). It is thus useful to normalize environmental situations when performing HRV analysis. In this respect, as we discussed earlier, acquiring a baseline of each ECG signals during a stabilization period is a necessary good practice to ensure correctness of execution and reproducibility of findings.

Consideration on Statistical Distribution and Tests

Statistical tests play a fundamental role in the entire process of a research study and for this reason we suggest that a dedicated statistical section should be always included in each ON empirical paper, regardless of the specific neuroscience method used.

A few considerations are worth noting for our scope here. HRV measures are not always normally distributed; therefore, their normality should be checked before using any test. Particularly, the frequency domain measures are non-normally and asymmetrically distributed, thereby requiring the use of non-parametric statistical tests. Among others, the non-parametric Wilcoxon Signed-Rank Test (Rey & Neuhäuser, 2011), which is also implemented in the majority of HRV software available, offers reasonable results. In case of multiple hypotheses testing, P-value adjustment is also used to avoid type I statistical error (for an application see Treister, Kliger, Zuckerman, Aryeh, & Eisenberg, 2012). Some authors also apply complex mathematical transformations to HRV non-Gaussian measures; this, however, introduces a degree of uncertainty that may produce less solid results (Castaldo et al., 2015)[9].

Moreover, due to individual biases and non-normal distributions, bootstrapping procedures and permutation tests may represent useful avenues to reduce the possibility of false-positive findings. For example, McCraty, Atkinson, and Bradle (2004), in investigating intuitive perceptions of

emotions with HRV analysis, used permutation to determine statistical significance of the differences between emotional arousing vs. controls. In more complex cases, advanced permutation entropy can also be used because it provides an advantageous complexity estimation leading to measures of the difference between two probability distributions (e.g., the Kullback–Leibler entropy).

Note that in social and behavioral research these procedures are often employed when investigators are utilizing HRV signals previously acquired for different purposes and should therefore be used with caution. In our experience, properly estimating the sample size is the most effective way to manage HRV intra-subject and intra-group heterogeneity and thus reduce errors. Generally speaking, organizational researchers should keep in mind that statistical significance increases with the number of subjects enrolled in a study and with the magnitude of the difference between the mean values of HRV measures in two conditions (or groups) under observation. Conversely, significance decreases with huge data dispersion.

Practicalities and Novelties

Tools, Costs, and Maintenance

The equipment and maintenance costs involved in performing HRV analysis are relatively small. As a ballpark figure, an excellent portable ECG recording device can be presently purchased for around $800. Its maintenance is also relatively cheap, requiring only batteries, memory cards to record data, and electrodes, which are single-use (1,000 electrodes costs roughly $100).

While there are several tools currently available in the market to optimize ECG data acquisition, we suggest use of instruments characterized by, at least, acquisition of 120 samples per second, and a resolution of 8 bit (i.e., 256 possible levels in the ECG amplitude measured in mV over a range of ±5 mV [better if ±10 mV]) (see also Task Force, 1996).

Thanks to novel technological developments, there has been an increasing availability of portable and wearable devices. Unobtrusiveness and wearability have brought forward, for instance, cardiac patches, smart t-shirts, skin sensors, and also electro-dermal printed circuits able to record reliable ECG data (Kyung Yang et al., 2008). In particular, recent advances in networking, data fusion, and sensing have gradually enhanced the potential of these devices for ON. For one, wireless connectivity jointly with the internet infrastructure, allows provision of real-time data. Moreover,

miniaturization of physiological trackers has led to increased computational and storage performance and low-power consumption for longer-term usage. These features have opened the opportunity to investigate real-life scenarios also enabling ubiquitous monitoring of research subjects.

Another promising advantage of these tools is linked to technologies of ambient intelligence (i.e., electronic environments that are sensitive and responsive to the presence of people). Physiological sensors can be integrated into clothing and also in the environment, so that information on heart rate variability can be captured in daily living. For instance, research has proposed video/thermal cameras and optical nanoscale sensing fibers embedded in objects of daily use, such as mugs or handles, as effective means to record cardiovascular activity (Ouwerkerk, Pasveer, & Engin, 2006). Building on this progress, technological evolutions related to HRV analysis can soon offer novel scenarios for investigations based on simultaneous observation and assessment of behavioral and physiological monitoring in real-life organizational settings. Indeed, these development and sensing techniques have increased the ecological validity of HRV measurements by minimizing alteration of experimental observations and represent some the most exciting applications of HRV analysis for organizational and ON research.

The use of novel physiological trackers also has possible drawbacks. For instance, long-term usability and comfort are aspects that are largely tool-dependent and may not always encounter the favor of researchers or subjects. Moreover, because of the ability to use wearable devices for long periods of time (such as overnight or over multiple days), the resulting data amounts might rapidly become overwhelming and require unexpected computational needs (i.e., “big data” approaches and processors).

In parallel, the literature has also expressed some concerns in relation to the use of HRV metrics derived from commercially available physiological trackers, like smart or sport watches (for a discussion, see Pagani et al., 2012). These remarks often tackle the fact that such devices use methodologies that may not guarantee an accurate assessment of the RR series. Indeed, they often rely on unclearly defined or proprietary (i.e., non accessible to researchers) algorithms to extract HR and autonomic indexes, even when they claim access to raw data. Similar concerns can be extended to other devices currently employed in and advocated for ON, like cheap EEG caps and basic eye-

tracking tools. For this reason, we suggest that the accuracy of the research tool used, the employment of purposely-designed codes, scoring data manually, and establishing laboratory protocols are all fundamental aspects to ensure the plausibility of findings and measures in ON research (see also Mendes, 2009).

Software

In aid of ON researchers, there are currently several reliable packages available to perform accurate HRV analysis in platforms like Matlab© and R©. For instance, ARTiiFACT (Kaufmann, Sütterlin, Schulz, & Vögele, 2011), which is based on MATLAB©, or RHRV (Rodríguez-Liñares et al., 2011), which is based on R©, are particularly relevant for behavioral research at large. Without gainsaying other reliable packages and platforms, the main advantage of these packages is that they can be easily integrated with other analytical tools assessing other physiological signals, like BOLD signals in fMRI research. Moreover, several accurate open-access tools have been recently developed (e.g., Singh & Bharti, 2015), such as PhysioToolkit© (Goldberger et al., 2000) and Kubios©, which is an intuitive platform allowing HRV analysis on all its main domains (Tarvainen et al., 2009). Online analytical tools, accessible via web browsers or as apps in smartphones and tablets, which enable sophisticate HRV analysis, may also represent useful instruments to further organizational neuroscience research (Melillo et al., 2015). Indeed, this development represents a promising avenue for undertaking research in real-world settings, involving employees and managers or using feedback protocols.

Ethical and Legal Concerns

Speaking generally, methods advocated for and employed in ON were primarily conceived for clinical applications and were only later applied to behavioral and social neuroscience research. This implies that these methods can prospectively inform pathological insights (Grossman and Bernat 2004), and lead to incidental findings—observations unexpectedly discovered in healthy subjects recruited to a research and unrelated to the purpose of the study (Illes et al., 2006). Therefore, the use of neuroscience methods in organizational research requires important considerations and precautions to safeguard both research subjects and researchers.

Specifically, HR-based analyses are regularly used in clinical practice and research; therefore, they are particularly exposed to the possibility of detecting pathological conditions or abnormalities. For instance, HRV analysis is a predictor of subjects at high risk after acute infarction (Malik & Camm, 1994) and in some specific instances can also be a prognostic predictor of mortality (Cole, Blackstone, Pashkow, Snader, & Lauer, 1999). Thus, because such information may also be uncovered during ON research, precise guidelines and protocols to deal with incidental findings should be well established before engaging in the research. These will necessarily have to adhere to best practices currently available and receive prior approval of deputy Ethical Research Boards (see also Task Force, 1996). Toward this end, it is worth keeping in mind that several international guidelines already exist to guide research toward protection of research subjects (e.g., Belmont Report, 1979). These indications are structured around promoting the subjects' rights to autonomy, dignity, and to not be misled or enrolled in a research without prior consent.

A related ethical concern focuses on the research subjects' privacy. With the increasing popularity of "commercial" physiological trackers, the possible exploitation of bio-physiological data acquired with commercial toolkits by the manufacturers has recently promoted a lively scholarly conversation (e.g., Morabito, 2016). Moreover, as Michael and Michael (2007) argue, the opportunity to ubiquitously assess individuals has often been confounded with an "opportunity toward omniscience," with resulting concerns about the privacy of the research participants, as well as possible issues of misinterpretation and misinformation.

Due to its early stage, ON seems to be particularly exposed to these latter concerns. Indeed, there are critical deontological considerations related to how untrained or inexperienced scholars may approach neuroscience research, particularly when communicating with participants and presenting findings in publications (e.g., Illes & Bird, 2006). Therefore, we strongly recommend the overall ON scholarly community engage further with disciplinary experts; always ensure adherence to the best available practices, including full methodological disclosure, rigor, and transparency; and possibly refrain from presenting suggestive but scientifically unsubstantiated claims as theoretical or practical implications of research.

Discussion

We are confident that readers can now better appreciate the capability of HRV analysis to provide quantitative information on the activity of the ANS (see Bechara et al., 1997). Yet, as hinted throughout the paper, a key issue related to the benefits of HRV analysis for management research remains. This regards the kind of information on organizational phenomena we can infer from HRV measures.

To shed a light on this crucial aspect, we now illustrate some theoretical and practical evidence useful to narrow the gap between extant research on HRV and organizational studies. Subsequently, we discuss crucial matters for ON regarding the level of inference obtainable in HRV analysis and, more generally, neuroscience research.

HRV Analysis and Organizational Research

Emotions. Recently, there has been an upsurge in interest in the study of emotions in organizations (e.g., Elfenbein, 2007). For instance, a better understanding of organizational actors' affective states is an important aspect for companies (Barsade & Gibson, 2007) and emotion regulation is vital to employee wellbeing and job performance (Côté & Morgan, 2002; Goldberg & Grandey, 2007). In parallel, a growing body of work has also begun to discuss how neuroscience methods can advance managerial research on emotions (for a review see Massaro, 2014).

Specifically, HRV analysis is emerging as an important tool to assess emotional regulation (for a review see Appelhans & Luecken, 2006). Emotional regulation strongly depends on a person's ability to alter their physiological arousal on an instant basis (Gross, 1998), thereby being well-suited for HRV analysis. In this respect, two main theories have focused on how HRV analysis can enrich our understanding of the psychological-physiological relationship of emotional regulation.

One is the polyvagal theory, which is developed from an evolutionary framework and contends that our ANS evolved in stages, each categorized by the development of an autonomic configuration that performs a specific role in social processes (Porges, 1997; 2001). This theory postulates that the ability of our vagal system to rapidly withdraw its inhibitory influence allows people to rapidly interact with their environments without requiring the slower sympathetic system. The nature of many organizational processes (e.g., dyadic exchanges, team communication, and leader-member exchanges) requires this rapid reaction. Thus, when the vagal withdrawal is

insufficient to meet the demands of the social environment, other autonomic subsystems can take place. Importantly for our purpose, this theory highlights the relation between indexes of ventral vagal system activity (i.e., arrhythmic responses, also measureable in terms of HRV) and the regulation of the emotional processes in social behavior (Porges, 2001).

On the other hand, Thayer and Lane (2000) have advanced a model of neurovisceral integration, which relates emotional responding to HRV specifically. These authors suggest that the multiple behavioral and physiological processes involved in emotions are just parts of a more complex structure. Our psychological states develop from interactions among lower level elements of the ANS, along dimensions of emotional valence and arousal. This framework contends that the central ANS is one of our "executive centers," governing our behavioral and physiological elements into regulated emotional states by inhibiting other physiological responses (Hagemann, Waldstein, & Thayer, 2003; Thayer & Seigle, 2002). Such inhibition would then be mediated both synaptically in the brain and in the periphery through vagal action (Thayer & Friedman, 2002). While there is vibrant debate on this matter, Thayer and Lane (2000) strongly argue that HRV helps assess the ability of the ANS to regulate the timing and magnitude of an emotional response.

Thus, as Appelhans and Luecken (2006) summarize, both the polyvagal and the neurovisceral integration perspectives are similar in that they both suggest a critical role for parasympathetic inhibition of autonomic arousal, and HRV is highly informative about people's capacity of regulating their emotional responding. We believe that these insights can be particularly salient to inform and refine organizational theory too.

For instance, these theories and HRV analysis can further our current understanding of the mechanisms of emotional contagion in organizations. Simply put, emotional contagion is the idea that people synchronize their emotions with those expressed by those around them. For instance, Barsade (2002) showed that groups in which a peer spreads a positive emotion showed an increase in positive mood, displayed more cooperation, and had less interpersonal conflict and better resource allocation.

In this respect, together with novel methodological opportunities (see Valenza et al., 2014), a simple assessment of an individual's emotional responses based on HRV computations may soon provide a more targeted understanding of the subjective mechanisms of emotional contagion. This can

in turn possibly offer novel feedback strategies based on human physiology to either enhance or limit a person's susceptibility to emotional contagion, thereby improving interpersonal relations, shared decision making, and cooperative environments in organizations.

Stress and burnout. HRV analysis has also been widely used to assess stress, which is another central construct in organizational theory and research (e.g., Schuler, 1980; Sosik & Godshalk, 2000). For one, it is common knowledge that mental stress reduces performance, both in the workplace as well as in everyday life.

The association between stress and HRV should not be surprising given the essential features of the ANS sympathetic and parasympathetic activities in responding to stressful or demanding external and internal situations. Importantly, HRV is widely acknowledged as a physiological index of stress because it entails a defined one-to-one relationship between the signal variations and the psychological state of a subject. For instance, research has shown that mental stress in work tasks implies a reduction in the HF component of HRV and an increase in the low- to high-frequency ratio compared to control situations (Hjortskov et al., 2004). Moreover, Castaldo et al., (2015) have recently offered a comprehensive meta-analysis on trends and the pivot values of HRV measures during mental stress. Likewise, recent advances in HRV analysis have begun to disclose that non-linear HRV analysis using short-term ECG recording could be effective in automatically detecting real-life stress condition (Melillo et al., 2011).

Because conflicting results concerning different HRV indexes exist, these features are typically more reliable at the group level. That is, it is challenging to predict if an individual participant is stressed or not. Despite being complex, detecting if an individual subject is under stress through HRV analysis can still be done. This however requires a study design with repeated experiments, in which the same subject is exposed to the same stressor several times and, possibly, over different days. The averaged HRV analysis over different repetitions can then be computed as the "typical" reaction of the individual to the stressor (pending no habituation).

Finally, despite still being at its early stage, HRV research has also proven useful to investigate burnout (Morgan, Cho, Hazlett, Coric, & Morgan, 2002; van Doornen et al., 2009), another important construct affecting and influencing many organizational behaviors (e.g.,

Brotheridge & Grandey, 2002; González-Romá, Schaufeli, Bakker, & Lloret, 2006).

Mental load and cognitive constructs. Research has also associated HRV with cognitive dimensions of the flow state associated with a task. For example, these measures have been used to infer a subject's mental load (de Manzano, Theorell, Harmat, & Ullén, 2010; Keller, Bless, Blomann, & Kleinböhl, 2011).

For instance, Taelman and colleagues (2011) performed an experiment in which subjects were exposed to three active conditions: a task with low mental load and a task with high mental load performed twice; each followed by a rest condition. They found that HRV measures could differentiate the active conditions from the resting conditions, indicating that HRV is sensitive to changes in mental states. Keeping in mind the consideration related to a within-subject research design, the authors showed that cognitive load decreases HF power and causes a shift toward a higher instantaneous frequency in the HF band.

Furthermore, research on mental load and HRV can benefit a wide-ranging set of research focusing on both "low-level" and "higher-order" cognitive constructs, such as mindfulness, goal setting, and attention, as well as memory, wisdom, and social dilemma. As an illustrative example, research focusing on the ANS has already shed a light on the vagal influence on working memory and attention (Hansen, Johnsen, & Thayer, 2003), thus providing room for future management research on this topic.

Moreover, Grossman and colleagues (Grossmann, Sahdra, & Ciarrochi, 2016) have recently shown that six HRV indexes can be positively correlated to wiser reasoning and less biased judgments when people adopt a self-distanced perspective as compared to a self-immersed one. Self-distancing allows individuals with higher HRV patterns to overcome egocentric impulses and reason wisely. Importantly, when research subjects were asked to relate to a person performing morally ambiguous actions, in the self-distanced condition, HRV indicators were positively related to occurrence of wisdom. Yet, this was not observed in the self-immersed condition. These findings resonate with recent research in ON and business ethics, which has begun to elucidate that the mechanisms of morally equivocal actions rely on one's empathy for others (Cropanzano et al., 2016).

Personality and individual differences. Personality and individual differences are other important research topics in organizational studies that can benefit from HRV analysis. For instance, it is well-known that differences in personality can explain performance and motivation in organizations' and employees' approaches to tasks (for a meta-analysis, see e.g, Judge & Ilies, 2002; for a theoretical account, see Motowildo, Borman, & Schmit, 1997).

Moreover, personality and HRV are each strong predictors of well-being. People's well-being is a significant aspect of human life, both inside and outside organizations (Edwards, 1992; Diener, 2000). Zohar and colleagues (2013) explored how autonomic regulation may mediate the development and maintenance of well-being in over 200 volunteers on a 24-hours HRV study. These authors found that openness, aggression, avoidant attachment, and forgiveness were found to positively and significantly relate to distinct HRV variables.

As discussed, ANS functioning is also associated with gender differences. Huang et al. (2013) showed that in 60 volunteers (30 males and 30 females), fatigability and asthenia were negatively correlated with HRV LF, HF, and total power (TP). Instead, novelty seeking was positively correlated with LF and TP. Further analyses revealed that the interactions "exploratory excitability x gender and fatigability x gender" predict LF and HF power respectively, suggesting that gender moderates the association between personality and ANS functioning. These insights may help refine and enrich business frameworks investigating personality characteristics and gender differences in e.g., entrepreneurs (e.g., Sexton & Bowman-Upton, 1990) or financial risk-takers (e.g., Powell & Ansic, 1997).

Behavioral monitoring and change. HRV analysis holds a number of implications for management practice. For instance, there is growing evidence of opportunities for behavioral monitoring and change, namely neurofeedback training. This protocol aims to teach people how to change their tonic level of physiological arousal by modulating their own HR responses (Lehrer et al., 2006; McCraty, 2005). These forms of neurofeedback applications may offer powerful advantages to interventions aimed at performance enhancement and leadership, as well as to coaching programs.

Indeed, encouraged by clinical evidence, neurofeedback represents one of the most promising opportunities to translate ON research into real-world practice (e.g., Waldman et al., 2011). However,

since research on neurofeedback in the workplace is fairly recent and still in need of further validation on its long-term effects, we believe it is important to highlight that neurofeedback in ON should be best understood and practiced as one component of more holistic behavioral change programs, rather than a one-stop definite intervention (Massaro, 2015).

Research Implications

HRV analysis can be applied to several domains of organizational and management theory and practice. Rather than advocating for incorporation of HRV analysis in each and every management study, here we wish to emphasize that a priority for future ON research will be to jointly ensure methodological and neuroscience accuracy and to clearly establish the level of inference involved in a study (cf. Cacioppo et al., 2007).

Throughout this work and specifically in the section "Inferring Information from HRV Analysis," we have provided a methodological description on how it is possible to extrapolate information on the ANS activity from HRV signals. However, it is also central for ON empirical research to determine the extent to which neuroscience measures can predict the occurrence and/or the variation of a psychological state or behavior in research subjects.

In this respect, scholars have suggested that the level of inference of a neuroscience experiment in the social sciences is determined by both the generality of the context in which the neurophysiological response occurs, and the specificity of this response in relation to the psychological state or behavior investigated (Cacioppo et al. 2007; Mendes, 2009). We believe that these are two important considerations useful to promote the theoretical advancement of the ON research program. Indeed, we trust that a key task for ON experimental research will be to disclose whether the psycho-neurophysiological relations investigated arise only in specific contexts (i.e., setting and/or population). Clearly, for ON to successfully develop as a field in its own right, research findings should strongly associate with definite and organizationally salient contexts (e.g., the workplace or a boardroom) and/or subjects (i.e., managers, leaders, entrepreneurs, and employees).

Toward this end, as explained throughout this work, research predictions will need to be validated with appropriate experimental controls (e.g., control samples or settings outside an organization) to ensure scientific rigor and appropriateness to the domain of organizational research

are maintained. Thanks to technological developments and its ease of use, HRV analysis seems particularly suited to address this need. Indeed, by enabling ambient sensing recording, offering portable devices, facilitating simultaneous investigation of multiple team members or dyads, this methodology allows the opportunity of forming novel ecologically valid and physiologically plausible organizational theories.

Moreover, given the abundance of organizational topics that can be investigated with neuroscience methods, the assessed physiological signals and neural correlates may have either one-to-one or many-to-one relationships with different psychological and behavioral constructs. In agreement with social neuroscience (Cacioppo et al., 2007), we suggest that this specificity interplays with the contextual dimension in that: (a) when the context is constrained, many-to-one relationships are referred to as outcomes; (b) when the context is defined, one-to-one relationships are referred to as markers; (c) when the context is generalized, many-to-one relationships are referred to as concomitants; and (d) when the context is unconstrained, one-to-one relationships are referred to as invariants. This classification is another important point for ON scholarship, which we trust is particularly suited to enrich knowledge on psycho-neuro-physiological outcomes and markers.

Overall, to precisely assess if a physiological response is a defined index for a psychological state or behavior, it will be necessary to proceed in a dynamic and multidisciplinary manner by disentangling experimental conditions, distinguishing between active and passive tasks, assessing context and specificity, and comparing such measures to established self-reported scales. This remark resonates with the importance of conceptualizing the use of neuroscience in organizational research as a research avenue that can complement, rather than replace, existing organizational research (Becker et al., 2011).

Keeping in mind these considerations, the use of ANS methods, including HRV analysis, will not only extend neuroscience investigations in management beyond the brain-level of analysis, but will also offer room for novel and intriguing research questions to arise. For instance, methodological research may focus on the best channels of neurophysiological activity to analyze group interactions; integrated ON research on the role of interoceptive awareness in representing conceptual knowledge

in the brain; and practical managerial research on the predictive power of HR fluctuations or HRV patterns as possible outcomes for leadership and coaching interventions in the workplace.

Conclusions

Recently, HRV analysis has emerged as an easy-to-use and valuable non-invasive methodology to assess ANS functions and allow inference on a number of constructs relevant for management. The combined gains of novel analytical methods, reliable and portable devices, and comprehensive software platforms suggest that this line of research will prosper in the future.

We seek to intrigue organizational researchers to join this promising research initiative. Thus, in this paper, we have reviewed and suggested several methodological and applicative insights, theoretical implications, and uses of HRV analysis in relation to organizational research and management practice. In conclusion, we hope that our effort will not only assist a wide-range of researchers in mastering this methodology—preventing procedural pitfalls and persevering scholarly rigor—but also will reinforce the idea that a multi-integrated and increasingly ecologically valid perspective on the entire nervous system is one of the most desirable, if not necessary, avenues to proficiently advance the ON research agenda.

Notes

[1]While acknowledging different views on organizational neuroscience present in the literature, with parsimony and inclusiveness in mind, here we refer to organizational neuroscience as that field using neuroscience theories, findings, practices, and/or methods to advance knowledge of organizational and management science, and vice versa.

[2]Given the complexity of the ANS, the available assessments of its activity are multiple, and there is not yet a single test that precisely reflects the function of a specific branch of this system. Here, by referring to ANS methods, we generally encompass advanced methods to assess heart-rate variability, baroreflex testing, and sweat output, which are primarily used for research purposes (Hilz & Dütsch, 2006). For the sake of completeness, other ANS methods involve the assessment of circulating catecholamines, microneurography, scintigraphic, and tests of autonomic reflexes, among others. These are generally used in clinical settings and are not routinely available.

[3]Together with the sympathetic and parasympathetic, a separate component of the ANS is the enteric nervous system (ENS), the intrinsic nervous system of the gastrointestinal tract (for a review, see Mayer, 2011). This system is relatively independent not only from the CNS but also from the other districts of the ANS.

[4]Vagal regulation is mediated through release of acetylcholine; the sympathetic regulation is mediated through epinephrine and norepinephrine. The acetylcholine promotes the response of muscarinic receptors, which in turn decrease the speed of the myocardium depolarization and promote decay in the heart rate. The epinephrine and norepinephrine have an opposite behavior; they activate the β-adrenergic receptors, resulting in acceleration of the myocardium depolarization and in higher heart rate. Note that this regulation mechanism does not have a direct effect on the nodes.

[5]Sometimes a U wave—of similar shape to a T wave but of lower amplitude—may follow the T wave. The U wave is usually seen in people with low heart rates and rarely when HR is high.

[6]During time intervals of various lengths, research subjects are asked to count their heartbeats silently without taking any reference (i.e., their pulse), and this measure is compared to their actual HR over the same time period in order to determine participants' heartbeat perception, often

computed as the mean error score of number and time of heartbeats between actual and perceived heartbeats.

[7]According to international guidelines (e.g., Task Force, 1996), HRV can describe both oscillations in the interval between consecutive heartbeats as well as oscillations between consecutive instantaneous heart rates.

[8]When a word related to a color is shown in a different color than the name, naming the color of the word takes longer and a greater subjective feeling of mental effort (i.e., high mental effort condition) for the subject, than when the name and the ink are congruent (i.e., low mental effort condition). For further methodological details and use of the test in HRV analysis see e.g., Castaldo, Melillo, & Pecchia, 2015; Hoshikawa & Yamamoto, 1997; for a review on the use of the test in strategy research, see also Lowett, 2005; for its use in mental effort research, see also Naccache et al., 2005.

[9]Note that the use of parametric tests on non-normally distributed measures is a relatively diffuse practice in HRV analyses to compare a study's results with previous findings in the literature. Yet, we caution that this approach often underestimates the number of subjects to be enrolled and/or overestimates the statistical power of the test.

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Tables

Table 1

Main ANS Sympathetic and Parasympathetic Stimulations

Structure	Sympathetic Stimulation	Parasympathetic Stimulation
Heart	Heart rate and force increased	Heart rate and force decreased
Iris (Eye muscle)	Pupil dilation	Pupil constriction
Salivary Glands	Saliva production reduced	Saliva production increased
Oral & Nasal Mucosa	Mucus production reduced	Mucus production increased
Lung	Bronchial muscle relaxed	Bronchial muscle contracted
Stomach	Peristalsis reduced	Gastric juice secreted; motility increased
Intestine	Motility reduced	Digestion increased (small); secretions and motility increased (large)
Kidney	Decreased urine secretion	Increased urine secretion

Table 2

Principal HRV Measures

Measure	Description	Unit
Time Domain		
AVNN	Average of all NN intervals	ms
SDNN	Standard deviation of all NN intervals.	ms
SDANN	Standard deviation of the averages of NN intervals in all five-minute segments of a 24-hour recording	ms
SDNN INDEX	Mean of the standard deviations of NN intervals in all five-minute segments of a 24-hour recording	ms
RMSSD	The square root of the mean of the sum of the squares of differences between adjacent NN intervals	ms
pNN50	Percentage of differences between adjacent NN intervals that are > 50 ms	%
pNN12	Percentage of differences between adjacent NN intervals that are > 12 ms	%
Frequency Domain		
TOTAL POWER	Total spectral power of all NN intervals up to 0.4 Hz.	ms^2
UVLF	Total spectral power of all NN intervals between 0 and 0.003 Hz	ms^2
VLF	Total spectral power of all NN intervals between 0.003 and 0.04 Hz	ms^2
LF	Total spectral power of all NN intervals between 0.04 and 0.15 Hz	ms^2
HF	Total spectral power of all NN intervals between 0.15 and 0.4 Hz	ms^2
LF/HF	Ratio of low to high frequency power	

Non-linear		
SD1	The standard deviation of the Poincare Plot (PP) perpendicular to the line of identity	ms
SD2	The standard deviation of the PP along to the line of identity	ms
En(0.2)	Approximate Entropy computed with the threshold r set to 0.2*SDNN	
En(rmax)	Approximate Entropy computed with the threshold r set to value which maximizes entropy	
D2	Correlation Dimension	
$\alpha 1$	Short term fluctuation slope in Detrended Fluctuation Analysis	
$\alpha 2$	Long-term fluctuation slope in Detrended Fluctuation Analysis	
lmean	Mean line length in RP	Beats
lmax	Maximum line length in RP	Beats
REC	Recurrence rate	%
DET	Determinism	%
ShEn	Shannon Entropy	

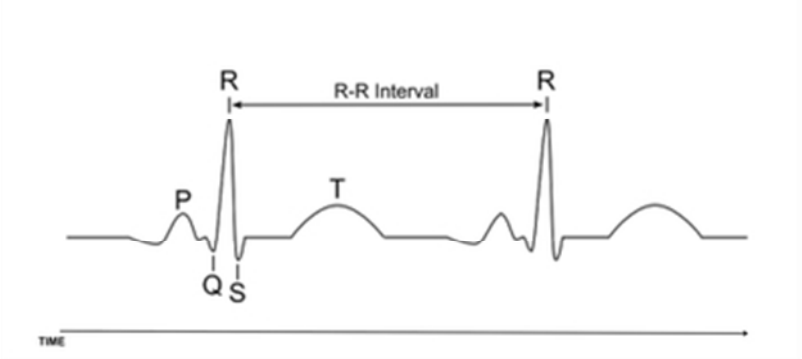

Figure 1: Standard EEG Waveform and RR Interval.

18x10mm (600 x 600 DPI)

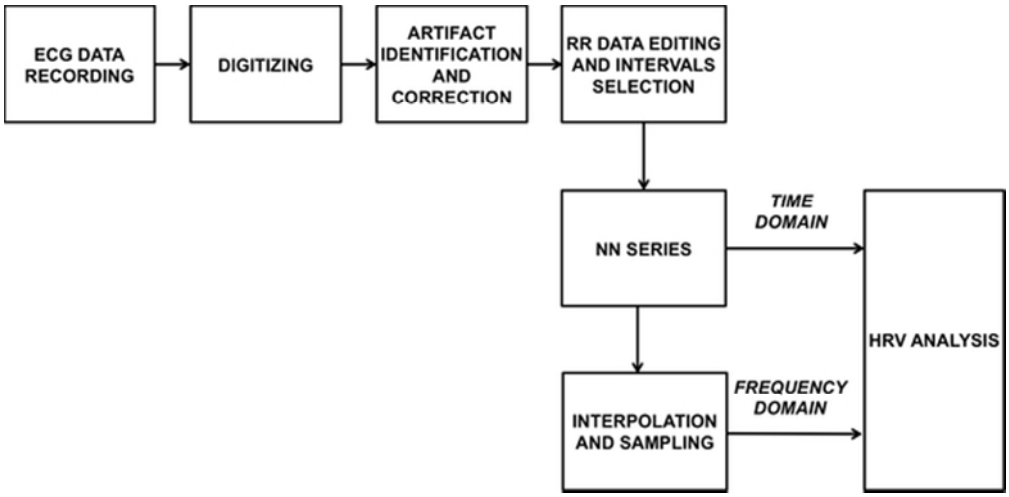

Figure 2: Breakdown of the Main Passages Used in HRV Analysis. Adapted from Task Force (1996).

50x24mm (300 x 300 DPI)

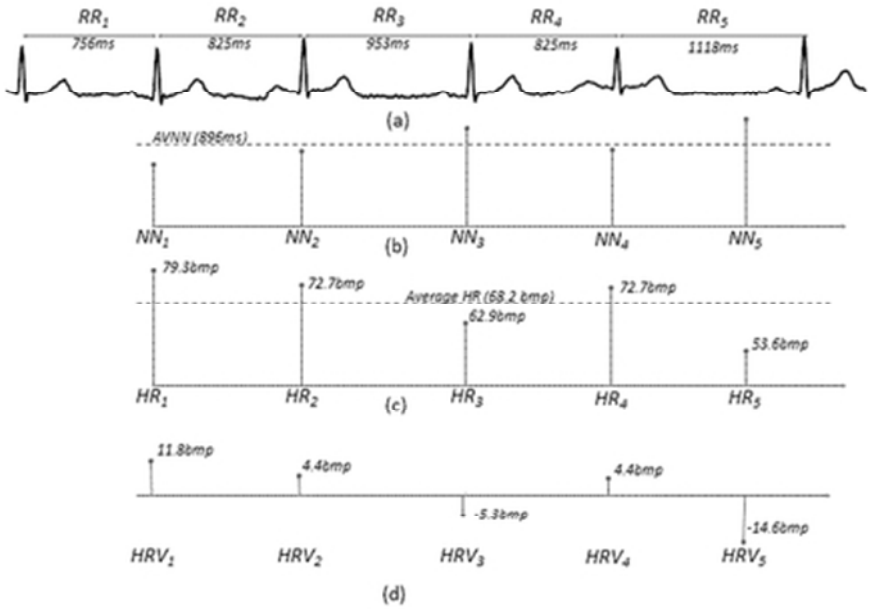

Figure 3: Illustrative Representation of HRV Extractions from an ECG Signal. (a) R peak detection, in which the R peaks are detected and the time interval among consecutive peaks (RR) is calculated; (b) peak annotation, in which heartbeats are qualified as normal and the NN series is generated; (c) NN series computing, in which iHR is calculated (iNN=1000 ms => iHR-60 bpm); and, d) HRV computing, in which the variation among consecutive heartbeats is computed (iHRV=iHR(n+1)-iHRn). The values reported indicate the bmp per each R peak through the different stages of analysis. Data:). Data: computed ad hoc for this work, available from the authors. Software: Kubios (Tarvainen, et al. 2009).

36x26mm (300 x 300 DPI)

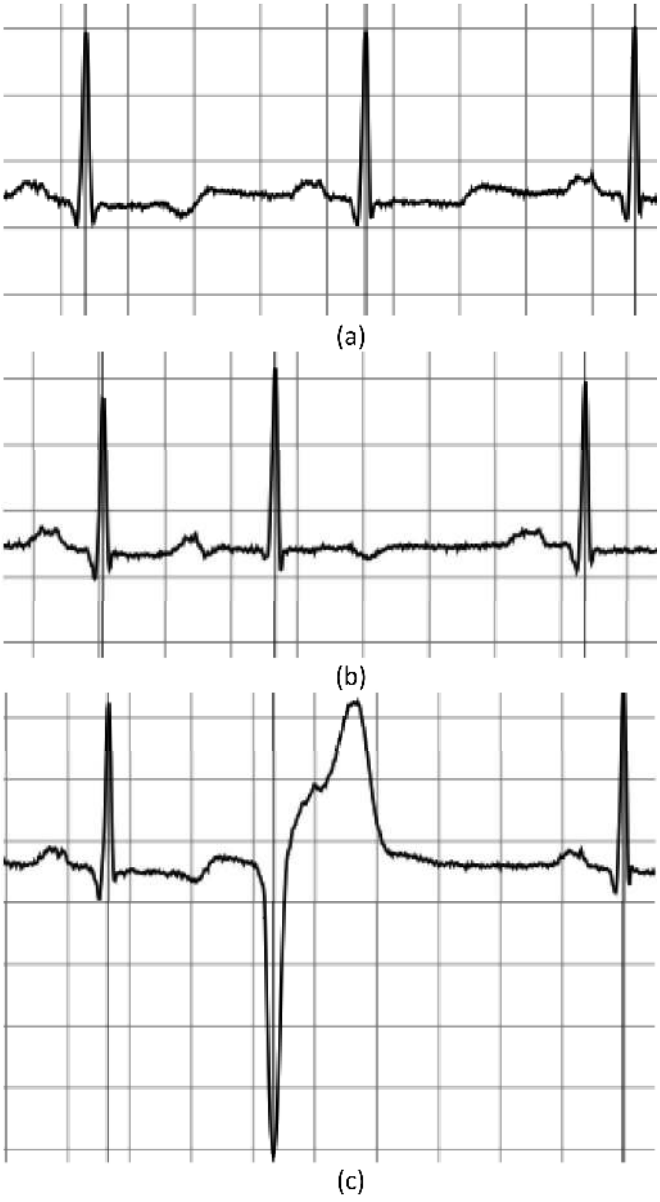

Figure 4: Normal and Abnormal Heartbeats. (a) normal heartbeats; (b) one normal heartbeat followed by one atrial premature heartbeat and then by an abnormal heartbeat; and, (c) one normal heartbeat followed by a ventricular premature heartbeat and then by an abnormal one. Data: Moody & Mark (2001); visualization tool: Goldberger et al. (2000).

222x408mm (300 x 300 DPI)

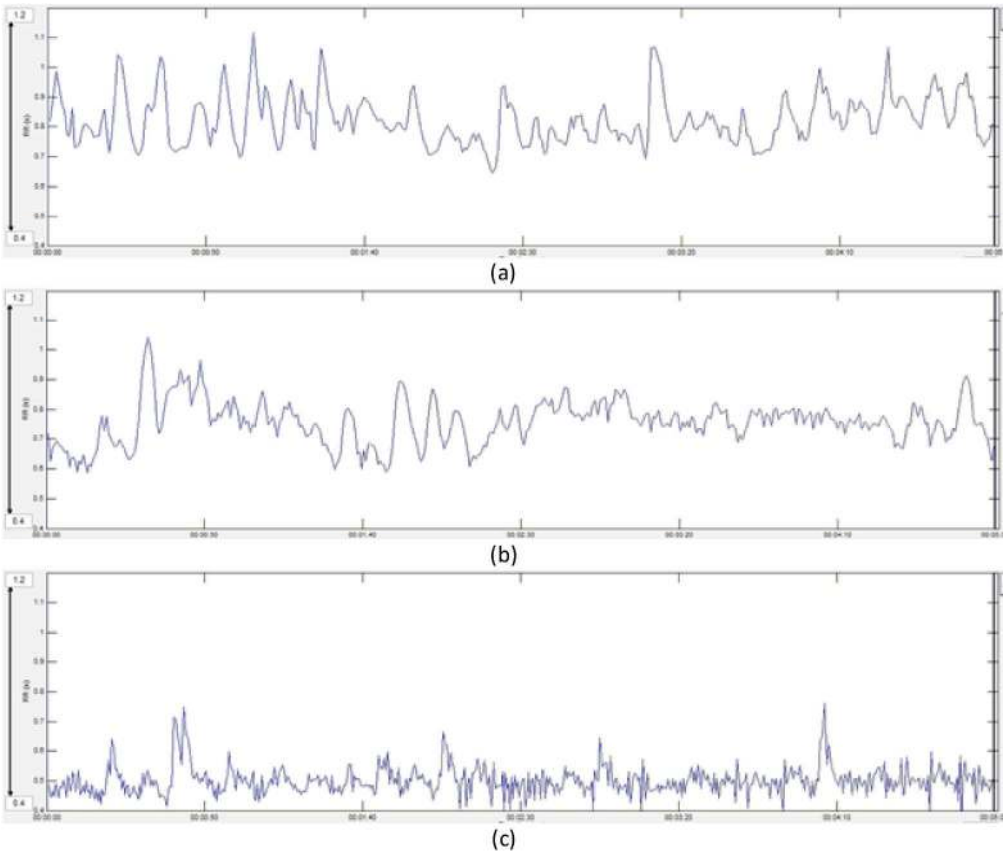

Figure 5: RR Series. We illustrate short-time RR series (five minutes) computed from the same subject: (a) in a resting condition; (b) when performing a low mental load task (congruent Stroop test); (c) when performing a more demanding mental load task (incongruent Stroop test). Note the depressed graph in (c) if compared to (b). Data: computed ad hoc for this work, available from the authors. Software: Kubios (Tarvainen, et al. 2009).

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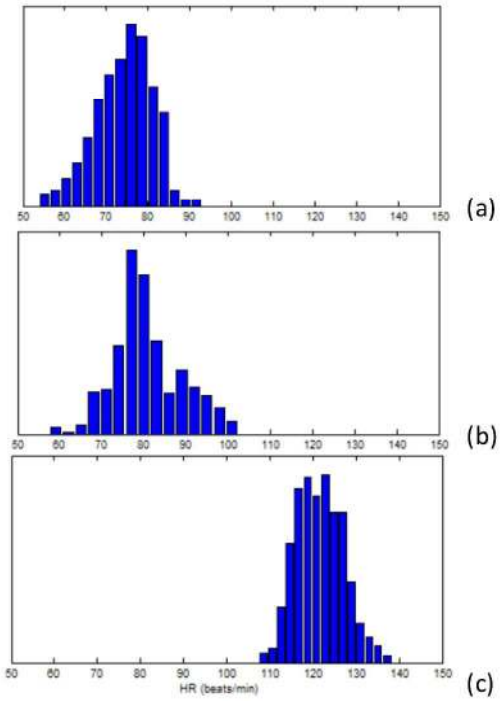

Variable	Units	Value
Mean RR*	(ms)	816.1
STD RR (SDNN)	(ms)	81.3
Mean HR*	(1/min)	74.20
STD HR	(1/min)	6.97
RMSSD	(ms)	52.0
NN50	(count)	91
pNN50	(%)	24.9
RR triangular index		15.913
TINN	(ms)	350.0

Variable	Units	Value
Mean RR*	(ms)	750.7
STD RR (SDNN)	(ms)	74.6
Mean HR*	(1/min)	80.72
STD HR	(1/min)	8.12
RMSSD	(ms)	35.9
NN50	(count)	61
pNN50	(%)	15.3
RR triangular index		13.759
TINN	(ms)	370.0

Variable	Units	Value
Mean RR*	(ms)	494.7
STD RR (SDNN)	(ms)	22.0
Mean HR*	(1/min)	121.52
STD HR	(1/min)	5.45
RMSSD	(ms)	18.9
NN50	(count)	9
pNN50	(%)	1.5
RR triangular index		7.805
TINN	(ms)	100.0

Figure 6: Histograms of the Heart Rate (HR) and Time Domain Measures. We illustrate the histograms of the heart rate and related time domain HRV measures computed from the same subject: (a) in a resting condition; (b) when performing a low mental load task (congruent Stroop test); (c) when performing a more demanding mental load task (incongruent Stroop test). Note in (c) there is a relevant increase of the HR (121±5 bpm), if compared to (b) in which the computed HR is 81±8 bmp. Note that the value of RMSSD, mostly related to parasympathetic activity, decreases from (b) to (c) with the increasing mental effort required by the task. Data: computed ad hoc for this work, available from the authors. Software: Kubios (Tarvainen, et al. 2009).

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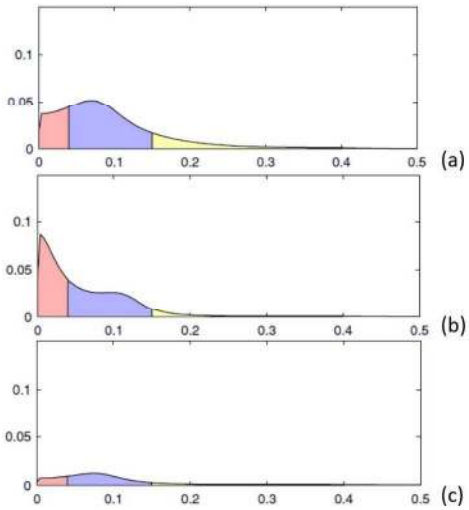

Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0391	1536	22.5	
LF (0.04–0.15 Hz)	0.0703	4222	61.9	79.9
HF (0.15–0.4 Hz)	0.1523	1062	15.6	20.1
Total		6821		
LF/HF		3.976		

Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0039	2518	44.6	
LF (0.04–0.15 Hz)	0.0430	2734	48.4	87.3
HF (0.15–0.4 Hz)	0.1523	397	7.0	12.7
Total		5650		
LF/HF		6.892		

Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF (0–0.04 Hz)	0.0391	310	20.2	
LF (0.04–0.15 Hz)	0.0742	935	61.0	76.5
HF (0.15–0.4 Hz)	0.1523	286	18.7	23.4
Total		1533		
LF/HF		3.270		

Figure 7: Power Spectrum and Frequency Domain Measures. We illustrate the power spectrum and related frequency domain HRV measures computed from the same subject: (a) in a resting condition; (b) when performing a low mental load task (congruent Stroop test); (c) when performing a more demanding mental load task (incongruent Stroop test). Note in (a) a regular spectrum with standard power; in (b) a depression of the power for the LF and HF bands; in (c) the spectrum is considerably depressed both as regards the total and the relative power of each frequency. Note the value of LF/HF ratio is similar in (a) and (c), suggesting caution in the interpretation of this value. Data: computed ad hoc for this work, available from the authors. Software: Kubios (Tarvainen, et al. 2009).

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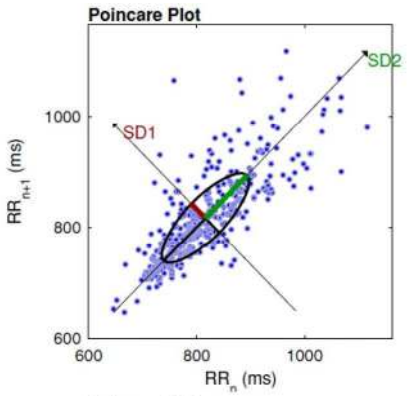
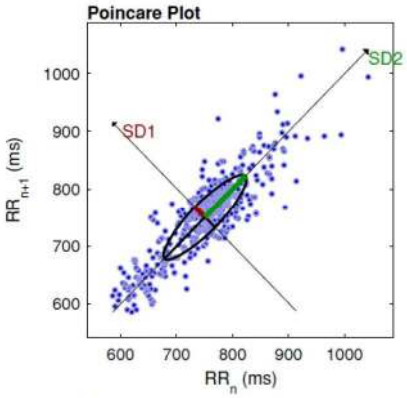
Variable	Units	Value
Poincare plot		
SD1	(ms)	36.8
SD2	(ms)	109.1
Recurrence plot		
Mean line length (Lmean)	(beats)	9.62
Max line length (Lmax)	(beats)	123
Recurrence rate (REC)	(%)	30.62
Determinism (DET)	(%)	98.56
Shannon Entropy (ShanEn)		3.034
Other		
Approximate entropy (ApEn)		1.028
Sample entropy (SampEn)		1.162
Detrended fluctuations (DFA): $\alpha 1$		1.479
Detrended fluctuations (DFA): $\alpha 2$		0.521
Correlation dimension (D2)		3.179
Multiscale entropy (MSE)		0.808 – 2.322

Variable	Units	Value
Poincare plot		
SD1	(ms)	25.4
SD2	(ms)	102.4
Recurrence plot		
Mean line length (Lmean)	(beats)	16.97
Max line length (Lmax)	(beats)	345
Recurrence rate (REC)	(%)	39.71
Determinism (DET)	(%)	99.21
Shannon Entropy (ShanEn)		3.568
Other		
Approximate entropy (ApEn)		1.105
Sample entropy (SampEn)		1.264
Detrended fluctuations (DFA): $\alpha 1$		1.601
Detrended fluctuations (DFA): $\alpha 2$		0.944
Correlation dimension (D2)		3.233
Multiscale entropy (MSE)		1.141 – 2.214

Variable	Units	Value
Poincare plot		
SD1	(ms)	13.3
SD2	(ms)	28.2
Recurrence plot		
Mean line length (Lmean)	(beats)	6.96
Max line length (Lmax)	(beats)	163
Recurrence rate (REC)	(%)	21.24
Determinism (DET)	(%)	95.94
Shannon Entropy (ShanEn)		2.677
Other		
Approximate entropy (ApEn)		1.378
Sample entropy (SampEn)		1.728
Detrended fluctuations (DFA): $\alpha 1$		0.963
Detrended fluctuations (DFA): $\alpha 2$		0.745
Correlation dimension (D2)		0.248
Multiscale entropy (MSE)		0.962 – 2.178



(a)



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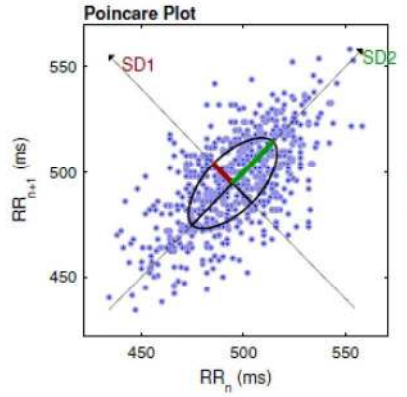

(c)

Figure 8: Poincaré Plot Analysis and Non-linear Measures. We illustrate the Poincaré plot analysis and related non-linear HRV measures computed from the same subject: (a) in a resting condition; (b) when performing a low mental load task (congruent Stroop test); (c) when performing a more demanding mental load task (incongruent Stroop test). During the intensive mental effort task, there is a significant reduction of the non-linear dynamics of HRV, as shown by decreased entropies values and correlation dimension, as well as by the shape of the plot in (c). Note that the values of SD1 and SD2 decrease as the demand of the mental effort task increases, indicating increased sympathetic modulation and parasympathetic withdrawal. Note that the references in the plots vary. Data: computed ad hoc for this work, available from the authors. Software: Kubios (Tarvainen, et al. 2009).

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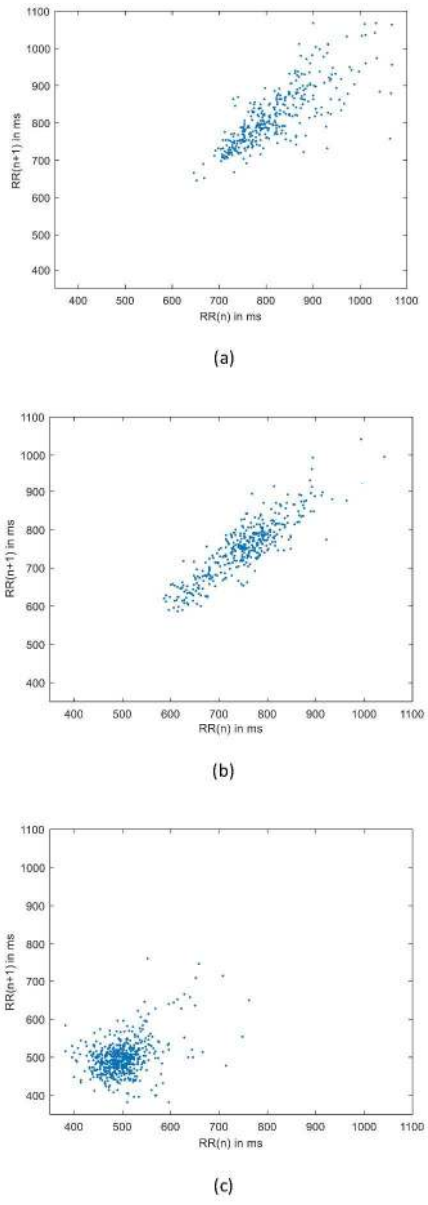

Figure 9: Poincaré Plot Analysis. We illustrate the Poincaré plot analysis computed from the same subject: (a) in a resting condition; (b) when performing a low mental load task (congruent Stroop test); (c) when performing a more demanding mental load task (incongruent Stroop test). The Poincaré plot analysis showed in Figure 8 is here computed on the same scales for all the three different conditions. Note the change in shape and magnitude in the plots. The points are more scattered when vagal activity offsets the sympathetic one as shown in (a) and (b); they are more concentrated when the sympathetic activity increases as shown in (c). Data: computed ad hoc for this work, available from the authors. Software: Matlab.

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Computational Details for HRV Analysis

We report mathematical details of some of the most used HRV features. This supplementary material complements Table 2 of the manuscript. Note, RR_j denotes the value of *j*'th RR interval and *N* is the total number of successive intervals

Time-domain HRV measures

Statistical measures.

- The simplest HRV feature is the mean value of the RR interval time-series, referred to as AVNN, and computed as:

$$AVNN = \frac{1}{N}\sum_{j=1}^{N} RR_j \quad \text{(Eq. 6)}$$

- The standard deviation of the RR intervals (SDNN) is:

$$SDNN = \sqrt{\frac{1}{N-1}\sum_{j=1}^{N}(RR_j - AVNN)^2} \quad \text{(Eq. 7)}$$

- The root mean square of successive differences (RMSSD) is:

$$RMSSD = \sqrt{\frac{1}{N-1}\sum_{j=1}^{N}(RR_{j+1} - RR_j)^2} \quad \text{(Eq. 8)}$$

- NN50, is another measure calculated from successive RR interval differences, computed as the number of successive intervals differing more than 50 ms and the corresponding relative amount pNN50 is:

$$pNN50 = \sqrt{\frac{1}{N-1}\sum_{j=1}^{N}\vartheta(\left|RR_{j+1} - RR_j\right| > 50\,ms)} \quad \text{(Eq. 9)}$$

where $\vartheta()$ is the Heaviside step function (i.e., the discontinuous function whose value is zero for negative argument and one for positive argument, formally $\vartheta(x) = 0$ if $x < 0$, otherwise $\vartheta(x) = 1$) and $|\ |$ is the absolute value operator.

- Conceptually alike is the pNN10, computed on 10 ms:

$$pNN10 = \sqrt{\frac{1}{N-1}\sum_{j=1}^{N}\vartheta(\left|RR_{j+1} - RR_j\right| > 10\,ms)} \quad \text{(Eq. 10)}$$

In long-term analysis, two other parameters are usually computed.

- SDANN, the standard deviation of the averages of NN intervals in 5-minute segments:

$$SDANN = \sqrt{\frac{1}{N-1}\sum_{j=1}^{N}(RR_{5j} - RR_5)^2} \qquad \text{(Eq. 11)}$$

- SDNN IDX, the mean of the standard deviations of NN intervals in 5-minute segments:

$$SDNN\ IDX = \frac{1}{N}\sum_{j=1}^{N} SDNN_{5j} \qquad \text{(Eq. 12)}$$

Geometric measures.

- HRV triangular index is the integral of the density distribution divided by the maximum of the density distribution.

$$HRV\ IDX = \int \frac{D(t)dt}{Y} \qquad \text{(Eq. 13)}$$

- TINN (ms) - Triangular interpolation of NN interval histogram, which corresponds to the baseline width of the density distribution measured through triangular interpolation. To compute TINN, it is necessary to select values *N* and *M* and a multi-linear function q such that q(t) = 0 for t ≤ N and t ≥ M and q(X) = Y, and such that the integral in Eq.14:

$$\int_0^{+\infty}(D(t) - q(t))^2\, dt \qquad \text{(Eq. 14)}$$

is the minimum among all selections of all values *N* and *M*. The TINN measure, expressed in ms, is:

$$TINN = M\text{-}N \qquad \text{(Eq. 15)}$$

Frequency-domain HRV measures

The frequency domain HRV measures rely on the estimation of power spectral density (PSD), which could be computed with several methods This estimation can be made with non-parametric and parametric methods. Fast Fourier Transform (FFT) computation is the basis of the non-parametric PSD analysis, as summarized in Table 2.

Nonlinear HRV measures

Poincare Plot. The parameters of the Poincarè Plot SD_1 and SD_2 are usually computed according to the following formulae:

$$SD_1 = \frac{SDSD}{\sqrt{2}} \qquad \text{(Eq. 16)}$$

$$SD_2 = \sqrt{2SDNN^2 - \frac{1}{2}SDSD^2} \qquad \text{(Eq. 17)}$$

where SDSD is the standard deviation of the difference of RR interval time series.

Approximate Entropy (AppEn). The AppEn is usually computed according to the following algorithm. A series of vector of length m $X_1, X_2, \ldots, X_{N-m+1}$ is constructed from the RR intervals as follows:

$$X_i=[RR_i, RR_{i+1} \ldots RR_{i+m-1}]. \qquad \text{(Eq. 18)}$$

The distance $d[X_i, X_j]$ between vectors X_i and X_j is defined as the maximum absolute difference between their respective scalar components. For each vector X_i, the relative number of vectors X_j for which $d[X_i, X_j] \le r$, $C_i^m(r)$ is computed where *r* is referred as a tolerance value (Eq. 19).

$$C_i^m(r) = \frac{number\ of\ \{d[X_i, X_j] \le r\}}{N-m+1} \quad \forall j \qquad \text{(Eq. 19)}$$

Then, the following index $\Phi^m(r)$ is computed by taking the natural logarithm of each $C_i^m(r)$ and averaging them over *i*.

$$\Phi^m(r) = \frac{1}{N-m+1} \sum_{i=1}^{N-m+1} \ln C_i^m(r) \qquad \text{(Eq. 20)}$$

Finally, the approximate entropy is calculated as:

$$ApEn(m,r,N) = \Phi^m(r) - \Phi^{m+1}(r) \quad \text{(Eq. 21)}$$

Either m=1 or 2, and r between 0.1 and 0.2 times the SDNN, are suitable values to compute AppEn.

Sample Entropy (SampEn). SampEn computation is similar to AppEn, but with two key differences: (a) in the computation of $C_i^m(r)$ the comparison of the vector X(i) with itself is included in the count for AppEn, while this comparison is excluded for SampEn; (b) the logarithm is applied instead of subtraction in the final step. These changes aims to remove the bias in AppEn, as the count of the self-comparison in AppEn lower its value and the signals are interpreted as more regular than they are. The steps of SampEn computation are described as follows:

$$C_i^m(r) = \frac{number\ of\{d[X_i, X_j] \le r\}}{N-m+1} \quad \forall j \neq i \quad \text{(Eq. 22)}$$

$$\Phi^m(r) = \frac{1}{N-m+1} \sum_{i=1}^{N-m+1} \ln C_i^m(r) \quad \text{(Eq. 23)}$$

$$SampEn(m,r,N) = \log \frac{\Phi^m(r)}{\Phi^{m+1}(r)} \quad \text{(Eq. 24)}$$

Correlation Dimension (CD). CD is computed similarly to AppEn. The reconstruction of the attractor is the first step to perform. That is, a series of vector of length m X_1, X_2, …, X_{N-m+1} is constructed from the RR intervals as follows:

$$X_i = [RR_i, RR_{i+\tau} \ldots RR_{i+\tau(m-1)}] \quad \text{(Eq. 25)}$$

where τ is the time delay and m is the embedding dimension. The second step is the estimation of Euclidean distances between each couple of vectors:

$$d[X_i, X_j] = \sqrt{\sum_{k=1}^{m} (X_i(k) - X_j(k))^2} \quad \text{(Eq. 26)}$$

Then, a function estimating the probability that two arbitrary points on the orbit are close than r is estimated. The correlation integral function is determined as:

$$C_m(r) = \frac{1}{N_m(N_m - 1)} \sum_{i}^{N_m} \sum_{j=1}^{N_m} \vartheta(r - d[X_i, X_j]) \qquad \text{(Eq. 27)}$$

where $N_m = N - \tau(m-1)$ and ϑ is the Heaviside function.

The correlation dimension is defined as the following limit value:

$$CD(m) = \lim_{r \to 0} \lim_{N \to \infty} \frac{\log C^m(r)}{\log r} \qquad \text{(Eq. 28)}$$

This limit value is approximated by the slope of the regression curve ($\log r$, $\log C^m(r)$). In HRV analysis the values of 1 and 10 are widely used value for τ and m, respectively.

Detrended Fluctuation Analysis (DFA). The DFA consists in the following steps:

a) The average $\overline{RR}$ of the RR interval series is calculated on all the N samples. The alternate component of the RR interval series, which is defined as the RR minus its average value $\overline{RR}$, is integrated:

$$y(k) = \sum_{j=1}^{k} (RR_j - \overline{RR}),\ k = 1,\ldots,N \qquad \text{(Eq. 29)}$$

b) The integrated series is divided into non-overlapping segments of equal length n. A least square line is fitted within each segment, representing the local trends with a broken line. This broken line is referred as $y_n(k)$, where n denotes the length of each segment.

c) The integrated time series is detrended as follows: $y(k) - y_n(k)$. The root-mean-square fluctuation of the detrended time series is computed according to the following:

$$F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^{N} (y(k) - y_n(k))^2} \qquad \text{(Eq. 30)}$$

d) The steps b) to d) are repeated for n from 4 to 64.

By representing the function F(n) in a log-log diagram, two parameters are computed: short-term fluctuations ($Alpha_1$) as the slope of the regression line relating $\log(F(n))$ to $\log(n)$ with n within 4-16; long-term fluctuations ($Alpha_2$) as the slope of the regression line relating $\log(F(n))$ to $\log(n)$ with n within 16-64.

Recurrence Plot. As in CD, vectors $X_i = (RR_i, RR_{i+\tau}, ..., RR_{i+(m-1)\tau})$, with $i=1,..., K$, with $K=[N-(m-1)\tau]$, where m is the embedding dimension and τ is the embedding lag, are defined. The recurrence plot is a K-dimensional matrix of dots, in which one dot is placed if the Euclidean distance between X_i and X_j is lower than a threshold value r.

The following steps are suggested in order to obtain the recurrence plot:

a) A K-dimensional square matrix M_1 is calculated computing the Euclidean distances of each vector X_i from all the others.

b) A K-dimensional square matrix M_2 is calculated as the matrix whose elements $M_2(i,j)$ are defined as:

$$M_2(i,j) = \begin{cases} 1 & if \quad M_1(i,j) < r \\ 0 & if \quad M_1(i,j) > r \end{cases} \qquad \text{(Eq. 31)}$$

The recurrence plot is the representation of the matrix M_2 in which a dot is associated to one value, that is, an image in which black pixels correspond to ones and white pixels to zeros. M_1 is a symmetrical matrix as the distance between X_i and X_j is equal to the one between X_j and X_i and consequently, the recurrence plot is a symmetric image along the diagonal. Usually the following values of the parameters are chosen: $m = 10$; $\tau = 1$; $r = \sqrt{m} * SDRR$.

In the recurrence plot, lines are defined as series of diagonally adjacent black points with no white space. The length l of a line is the number of points constituting the line. Measures of the recurrence plot are widely computed: recurrence rate (REC) defined in Eq. 32; maximal length of lines (l_{max}); mean length of lines (l_{mean}); the determinism (DET) defined in Eq. 33; the shannon entropy (ShEn) defined in Eq. 34.

$$REC = \frac{1}{K^2} \sum_{i=1}^{K} \sum_{j=1}^{K} M_2(i,j) \qquad \text{(Eq. 32)}$$

$$DET = \frac{\sum_{l=2}^{l_{max}} l * N_l}{\sum_{i=1}^{K} \sum_{j=1}^{K} M_2(i,j)} \qquad \text{(Eq. 33)}$$

with N_l = number of lines of length l

$$ShEn = \sum_{l=l_{\min}}^{l_{\max}} n_l * \ln n_l \qquad \text{(Eq. 34)}$$

with n_l = percentage of N_l over all the number of lines.